ECOLOGY AND EVOLUTION OF MALARIAL PARASITES IN VERTEBRATE HOSTS

A Dissertation

Presented to the Faculty of the Graduate School

Of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

By Holly Lynn Lutz

August 2016

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Cornell University 2016

This dissertation represents a culmination of extensive field work and collections of African vertebrates and their symbionts, as well as experimental studies carried out in the laboratory. Field work was conducted primarily in the East African countries of Kenya, Malawi, Mozambique, and Uganda. Throughout these expeditions, efforts were made to improve field protocols for the comprehensive sampling of wild vertebrates and their symbionts, with particular focus on the sampling of avian blood parasites (haematozoa), ectoparasites (arthropods), endoparasites (helminths), and microbial symbionts (bacteria and viruses). This dissertation therefore includes a chapter with detailed guidelines and protocols for sampling avian symbionts based on these experiences. Following chapters rely on data from both field collections and laboratory experiments, which provide a foundation for addressing the ecology, systematics, and molecular evolution of malarial parasites in vertebrate hosts. Specifically, haemosporidian data from 2,539 Afrotropical birds and small mammals (bats, rodents, and shrews) collected during field inventories were used to (1) test hypotheses linking host life history traits and host ecology to patterns of infection by three haemosporidian parasite genera in birds (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), and (2) re-evaluate the molecular phylogeny of the order Haemosporida by incorporating existing genomic data from haemosporidian parasites with data from novel parasite lineages infecting major vertebrate host groups, including birds, mammals,

and reptiles. In addition to these studies of host–parasite ecology and parasite phylogeny, this dissertation includes an experimental demonstration of the utility of laser microdissection microscopy techniques as a tool for acquiring the DNA of four avian parasites (*Plasmodium relictum*, *P. homocircumflexum*, *P. ashfordi*, and *P. cathemerium*) from nucleated red blood cells of avian hosts. Experimental data derived from the application of laser microdissection microscopy techniques were subsequently combined with genomic data provided by the Wellcome Trust Sanger Institute to examine the extent to which positive Darwinian selection has shaped the evolution of gene families involved in the invasion of host cells and evasion of host immune response by the avian malarial parasite, *Plasmodium relictum* (lineage pSGS1).

This dissertation improves our understanding of the effects of host ecology and life history on patterns of infection by haemosporidian parasites in Afrotropical birds. Life history traits such as nest type, nest height, and flocking behavior in birds have significant effects on the prevalence of malaria infection in vertebrate hosts. Phylogenetic analyses of novel parasite lineages, collected and identified from East African vertebrates, show that the evolutionary history of haemosporidian parasites involves host shifts between major vertebrate host groups, and that bats were likely the first mammals to acquire haemosporidian parasites from a sauropsid-infecting ancestor. Subsequent transitions from bats to other non-chiropteran mammals appear to have led to the extant diversity of haemosporidian parasites infecting primates and rodents. This phylogenetic hypothesis has important implications for the evolution of life history traits in haemosporidian parasites themselves, including the production of haemozoin pigment (a byproduct of haemoglobin digestion), and erythrocytic schizogony (asexual reproduction in host red blood cells).

BIOGRAPHICAL SKETCH

Holly Lutz was born in Altus, OK at the Altus Air Force Base. For the next five years, she moved between Oklahoma, North Dakota, and Texas, until settling in Fort Worth with her parents and siblings. Following three years of attending public schools, Holly was homeschooled for four years, after which she attended Nolan Catholic high school for grades 7–12. She began attending the University of Chicago in the fall of 2005, and received her BA in the Biological Sciences with a specialization in Ecology and Evolutionary Biology in 2009. During her time as an undergraduate, Holly worked in the marine science laboratories of Drs. Tim Wootton and Cathy Pfister, and spent several months as an assistant to Drs. Wootton, Pfister, and Robert Paine in the Pacific Northwest. There, she contributed to projects on Tatoosh Island and along the rocky intertidal of the Strait of Juan de Fuca. Following these experiences, she transitioned to working with the Field Museum of Natural History in Chicago, where she acquired extensive experience in bird and mammal specimen preparation, molecular lab work, and international field work. From 2009–2011, Holly worked as a member of the Emerging Pathogens Project at the Field Museum, completing three international expeditions to Africa and South America, and contributing to the collection of several thousand bird, mammal, and symbiont specimens for the Field Museum. It was during this time, and from examining these specimens, that Holly developed a keen interest in malarial parasites of wild birds and mammals. She carried this interest in malarial parasite ecology and evolution with her to Cornell in the fall of 2011, and it thus became the topic of her dissertation.

DEDICATION

To Elliot, Lauren, and Tycho

To allow mystery, which is to say to yourself, “There could be more, there could be things we don’t understand,” is not to damn knowledge. It is to take a wider view. It is to permit yourself an extraordinary freedom: someone else does not have to be wrong in order that you may be right.

- Barry Lopez (Of Wolves and Men)

ACKNOWLEDGEMENTS

My graduate committee has served as a great source of intellectual stimulation and academic guidance during my development as a scientist. I am incredibly grateful to my committee as a whole for allowing me to operate independently, while remaining available for guidance and support throughout the duration of my PhD. In particular, I thank Drs. André Dhondt and Michael Stanhope for co-chairing my committee and reviewing myriad drafts of manuscripts and proposals, always with constructive criticism, useful feedback, and great patience. I also thank Dr. Wesley Hochachka for his ever-present kindness, support, and patience as I navigated my PhD and developed my research ideas. Countless hours spent discussing general ideas or specific models helped me remain grounded and productive, particularly in the earliest stages of my dissertation. I am grateful to Dr. Irby Lovette for making available or drawing my attention to many important resources that have allowed me to effectively pursue my research. I am particularly grateful for his dedication to various aspects of ornithology-related activities, including the annual Ornithology Seminar series, and myriad Lab of Ornithology events in which he invited me to participate. Dr. Lovette's encouragement of my participation in such events has played a very important role in both my development as a scientific communicator, and in the development of my general knowledge of avian biology. Last but not least, I am grateful for the enthusiastic encouragement and support of Dr. John Fitzpatrick, without whom I might not have applied to the graduate program at Cornell. Through a chance encounter in Chicago, I discovered Dr. Fitzpatrick's shared passion for natural history and museums, which led me to apply for graduate study at Cornell. This understanding has

continually provided me with a strong sense of belonging in an academic setting that is incredibly diverse, and Dr. Fitzpatrick's encouragement of independence in his students has allowed me to pursue a scientific path which otherwise might have seemed unattainable.

I thank my parents for their selfless dedication to my early education, and my many friends around the world for their good humor and support as I've navigated my graduate program. My studies and research at Cornell were intertwined with my work at the Field Museum of Natural History in Chicago, and I would like to thank the many friends and 'family' at FMNH with whom I have worked over the years and during my PhD. Drs. Shannon Hackett, John Bates and Jason Weckstein were crucial in steering me towards a research career. Before meeting and working with them, I had not realized that research was an option, having come from a very different background. I am incredibly grateful for the friendship and support of Dr. Kevin Feldheim, Erica Zahnle, Brian Wray, and Josh Engel as I have worked my way through myriad laboratory, field, and life experiments. I thank Drs. Julian Kerbis, Bruce Patterson, Ben Marks, and Corrie Moreau for their friendship, mentorship, and support as I have pursued scientific-collecting and museum-based research. I will be forever grateful to Mary Hennen and Dr. Dave Willard for their patience, kindness, and friendship for as long as I had been a nuisance at the museum. Lastly, I would not be where I am as a scientist and field biologist without the guidance, encouragement, epic friendship and love of Thomas Gnoske, who permanently altered the course of my life on the day he taught me how to prepare a scientific specimen.

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CHAPTER 1

A BRIEF HISTORY OF THE STUDY OF MALARIA AND FUTURE DIRECTIONS FOR HAEMOSPORIDIAN RESEARCH

Introduction

Malaria parasites (Apicomplexa: Haemosporida) constitute a major global health threat to humans, causing over half a million deaths per year in developing regions of the world (World Health Organization World Malaria Report 2015). Yet, only six species in the genus *Plasmodium* are responsible for human malaria, while over two hundred species exploit terrestrial vertebrate wildlife (Garnham 1966; Valkiūnas 2005). The ecological factors driving host–parasite associations in wild animals are poorly studied in most regions of the world, and the evolutionary host-switches that have led to the infection of humans by malaria parasites continue to be re-evaluated in light of new discoveries by parasitologists. In addition to causing the extinction of species and mass mortality (Atkinson & LaPointe 2009; Samuel et al. 2015), malarial parasites have galvanized the creation of novel insect eradication methods (DDT, gene drive, etc.) (Greenwood et al. 2008; Hammond et al. 2016), and organizations dedicated to human health and zoonotic diseases. Indeed, the Centers for Disease Control (CDC, originally called the Communicable Disease Center) was created for the primary purpose of eradicating malaria in the United States (Etheridge 1992), continuing the efforts of the World War II "Malaria Control in War Areas" program in the Southern United Sates.

Technological advances made in the late twentieth and twenty-first centuries have opened new doors in the study of haemosporidian parasites, but progress in this field is merely continuing to build on a rich and storied past of haemosporidian research. The future of this field is also bright, with innumerable discoveries that may have direct implications for human health. Surveys of African vertebrates (Chasar et al. 2009; Loiseau et al. 2012; Sehgal et al. 2010), especially of African primates (Herbert et al. 2015; Prugnolle et al. 2010), have revealed a complex story of host-switching and parasite-sharing between hosts, and suggest the existence of potential new model organisms for laboratory research (Schaer et al. 2013). Malarial parasites in mammals, particularly those in the Old World tropics, remain largely unexplored, while recent surveys of avian hosts in these regions have revealed astonishing diversity. Indeed, diversity in birds appears to be greater than in mammals in most geographic regions (Lutz et al. 2015; Lutz et al. 2016). Thus, there remains a great deal to explore and describe with respect to malarial parasite diversity and host associations.

A brief history of the discovery of malaria and its etiology

Malaria (from Italian "*mala aria*" meaning "*bad air*") is perhaps one of the most ancient and well-studied diseases in human history, and yet, progress in preventing and treating malarial infection has been relatively slow, compared to other deadly human afflictions. Clinical symptoms of malaria have been documented as far back as the twenty-seventh century BCE by Hang-ti, the legendary Yellow Emperor of China, in the *Nei ching* (*The Canon of Medicine*), and by Ancient Egyptians in the fifteenth century (Breasted 1930; Garnham 1966; Halawani and Shawarby 1957) (Figure 1.1). By the fourth century, explicit details of malarial infection had

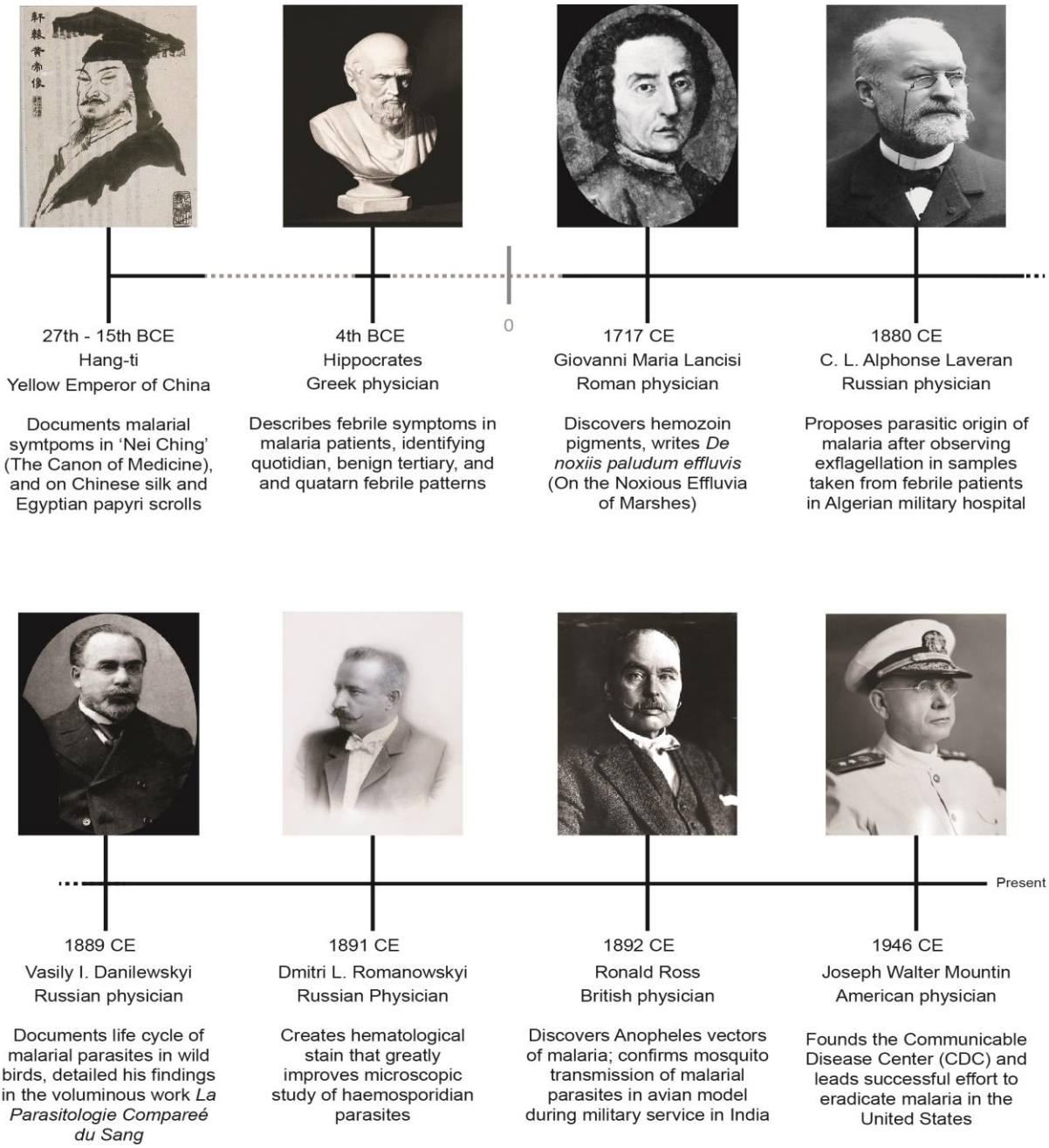

Figure 1.1. Timeline of significant events in the identification, research, and management of malaria (timeline not to scale).

been documented by the Greek physician Hippocrates, who described various patterns of febrile symptoms (quotidian, benign tertian, and quartan) brought on by the disease (Coxe 1846; Pappas et al. 2008). Despite this knowledge of the disease and a fairly universal development of treatments among various cultures (herbal *Artemisia annua*, aka wormwood, in China, quinine-

containing bark of *Cinchona* trees in South America, etc), it was not until the eighteenth century CE that the etiology of malaria began to be elucidated. The first clue to the disease's source came with the discovery of hemozoin pigment in human cells (hemozoin pigment is a byproduct of hemoglobin digestion by malarial parasites) by the Roman physician Lancisi in 1717 CE. This discovery was followed by the work of Charles Louis Alphonse Laveran, who focused his observations on febrile patients in military hospitals during the late nineteenth century. Based on his observations of the exflagellation process (Figure 1.2), Laveran deduced that malaria was parasitic in origin. His hypothesis, however, was highly criticized by contemporary scientists and physicians, who continued to believe that the disease must be of bacterial origin and transmitted through the air.

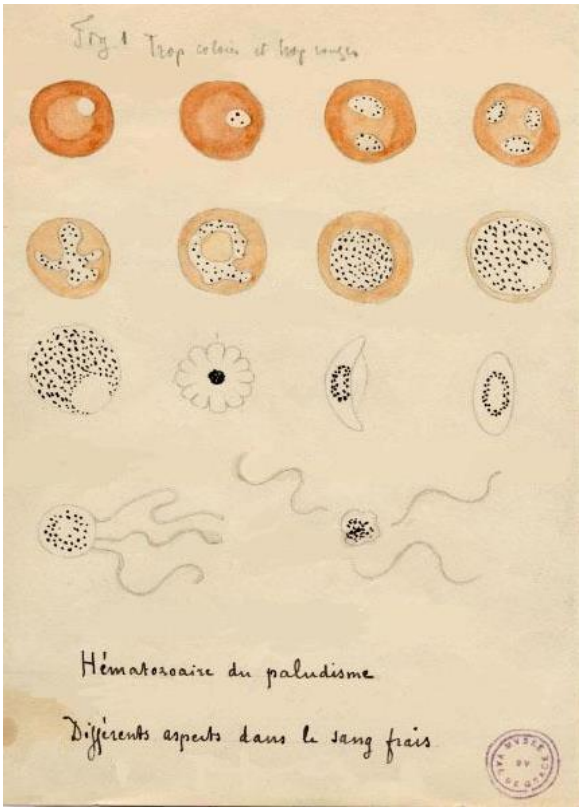

Figure 1.2. Original drawing by Charles Louis Alphonse Laveran of his discovery of the exflagellation stage of malarial parasite development. Exflagellation occurs when infected red blood cells experience changes in temperature and pH, indicative of a shift from the human to the mosquito host.

At approximately the same time that Laveran was making his discoveries, the Russian scientist Vasily Iakovlevich Danilewskyi was making independent observations of protozoan parasites in local birds. His observations were detailed and thorough, and described the nearly-complete life history of some avian parasites in the blood cells of infected birds, as well as the clinical effects these little-known parasites had on their hosts. Danilewskyi's research culminated in his voluminous work titled *La Parasitologie Compareé du Sang* (1889), which was the first major attempt to document the diversity and distribution of haematozoan parasites (haemogregarines, haemosporidians, etc) in wild animals. The study of malarial parasites in birds continued to play a prominent role in the advancement of knowledge as a series of technological

developments and discoveries were made by malariologists and pathologists (e.g., the Russian physician Dmitri Leonidovich Romanoswky's accidental discovery of oxidized methylene blue – known as "polychrome methylene blue" – which allowed parasite nuclei in blood films to be more easily visualized, and which is now included in most modern hematological stains, such as Giemsa, Wright's, etc.). The work of Laveran and Danilewskyi set the stage for the groundbreaking discovery by Sir Ronald Ross, a British physician, who found that malarial parasites are transmitted by mosquitoes (Figure 1.3). On the day of his discovery, Ross composed the following poem, which he sent to his wife:

This day relenting God
Hath placed within my hand
A wondrous thing; and God
Be praised. At His command,
Seeking His secret deeds
With tears and toiling breath,
I find thy cunning seeds,
O million-murdering Death.
I know this little thing
A myriad men will save.
O Death, where is thy sting?
Thy Victory, O Grave?

Following his discovery of the malarial vector, Ross pursued experiments using birds as models to demonstrate the full transmission cycle of malarial parasites, making many more detailed observations on the life history and morphology of the parasite as it transitioned through these life stages (Cox 2010). Ross's discovery heralded the dawn of a new era for mankind in the battle against malaria. Once it was discovered that mosquitoes are responsible for the transmission of

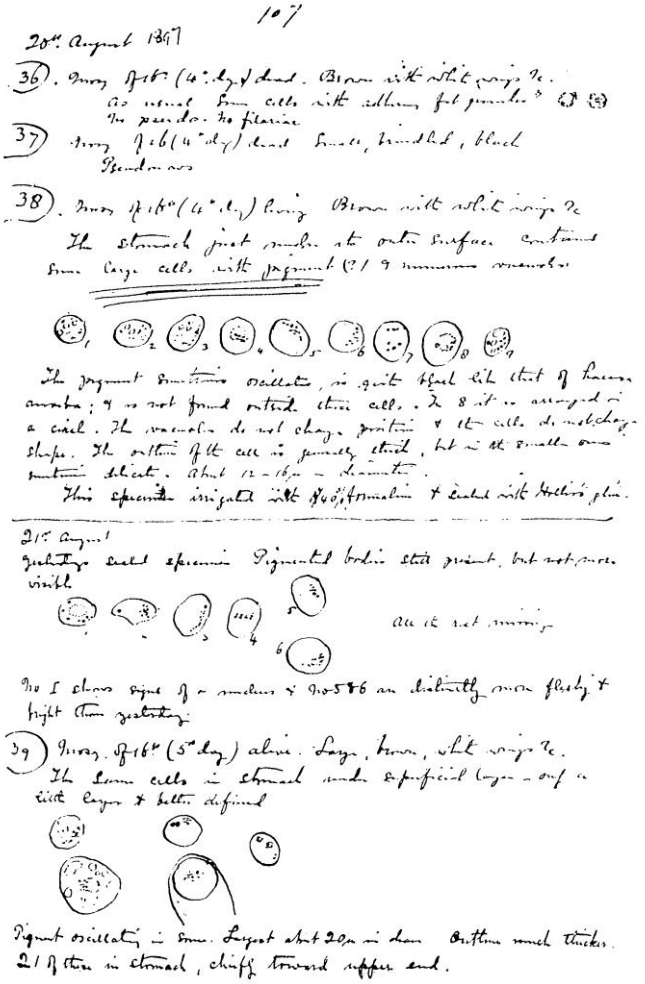

Figure 1.3. Page from the notebook of Ronald Ross, in which he describes his observations of malarial parsite development in the midgut of an infected mosquito.

malarial parasites to humans, great efforts were made to eradicate mosquitoes in developed regions of the world. Indeed, the United States of America founded the Communicable Disease Center (now known as the Centers for Disease Control and Prevention) in 1946 for the primary purpose

of curbing malaria cases by eradicating mosquitoes in the continental US, following the World War II "Malaria Control in War Areas" program run by the Office of National Defense Malaria Control Activities.

One of the next great advances for basic research of malarial parasites came during the late twentieth century with the development of molecular methods (e.g., PCR and Sanger sequencing) to specifically target regions of the haemosporidian genome for genetic analysis. The application of these methods led to many novel and conflicting hypotheses regarding the evolutionary relationships and taxonomy of known Plasmodium species. Most prominent of these was the "avian origin hypothesis," which suggested that the deadliest form of human-infecting Plasmodium, *Plasmodium falciparum*, shared a most recent common ancestor with the avian parasites, including a parasite found in domestic chickens, *Plasmodium gallinaceum* (Waters et al. 1991). This hypothesis seemed plausible given the high virulence and pathogenicity of *P. falciparum*, which was thought to be indicative of a recent host-transition. Additional genetic analyses also seemed to support this hypothesis (McCutchan et al. 1996). However, these studies were hindered by poor taxon sampling and possible methodological errors (e.g., selection of inappropriate genes or outgroups for phylogenetic analyses) (Perkins 2014). The avian origin hypothesis is now considered to be obsolete, as additional *Plasmodium* species and genes have been discovered and employed for phylogenetic analyses (Blanquart and Gascuel 2011; Cai et al. 2012; Lutz et al. 2016; Martinsen et al. 2008).

However, the haemosporidian phylogeny remains far from being "resolved", due in large part to the paucity of taxonomic sampling in many regions of the world. Since the discovery of haemosporidian parasites by Danilewskyi and Laveran in the late nineteenth century, few vertebrate species and geographic regions have been carefully explored for haemosporidian

diversity and host–parasite associations. The Old World tropics of Africa and Asia have been particularly neglected. Most recent surveys in these regions have revealed a large number of novel genetic lineages (defined by genetic variation in the mitochondrial cytochrome *b* gene), and these lineages likely correspond to reproductively isolated units (i.e., biological species) (Bensch et al. 2004). Continued exploration of parasite diversity in these regions of the world, as well as in the Neotropics and temperate zones in the Southern Hemisphere will likely to continue filling in major gaps in the haemosporidian Tree of Life. Even in regions that have been relatively well-explored, such as North America, new species are being discovered and molecularly characterized as sampling of broader host ranges continues. Most recently in North America, *Plasmodium* parasites were identified across a large range of white tailed deer (*Odocoileus virginianus*) populations across the Southern United States (Martinsen et al. 2016). The study found *Plasmodium* parasites to be well-established and genetically diverse in these deer populations, indicating a lengthy coevolutionary relationship between *Plasmodium* and deer hosts. Another recent study focusing on ungulates found *Plasmodium* parasites to be readily detectible in water buffalo (*Bubalis bubalis*) populations throughout their ranges in Vietnam and Thailand (Templeton et al. 2016). These discoveries beg the question: what else is out there?

Future directions in the study of haemosporidian parasitology

It is crucial to note that the source of malaria might never have been discovered were it not for the persistent efforts of curious researchers in their sampling of vertebrate and invertebrate haemosporidian hosts. Such persistence underlies many important discoveries linking symbiotic life forms (protozoan, bacterial, and otherwise) to longstanding, or emerging infectious diseases,

and in many cases, the study of symbionts has led to an explanation or improved understanding of adaptation or ecological shifts in host organisms (Whiteman & Parker, 2005). In essence, vertebrate hosts function as microcosmic ecosystems, providing resources and a platform for resource exchange between a plethora of microorganisms. To study a vertebrate organism without, at the very least, acknowledging the possible effects of symbiont diversity on its evolution, ecology, and behavior (and vice versa) is to neglect a large part of the biological picture. With this realization in mind, many vertebrate biologists have begun to consider symbionts in their research programs. Indeed, studies of haemosporidian parasites in birds have experienced a renaissance of sorts in the late twentieth and early twenty-first centuries, and have produced valuable insights into avian biology (e.g., Dunn et al. 2011; Lachish et al. 2011). Studies of ectoparasitic lice in primates have shed light on patterns of evolution and diversification (Johnson et al. 2014), and trypanosome surveys in East African rodents have led to the discovery of cryptic mouse species in rapidly disappearing forest patches (Salzer et al 2015). As the interest in sampling symbionts along with vertebrate hosts grows, so too does the need for sampling schemes that are both comprehensive and practical in field situations.

Thus, with the understanding that many important discoveries in the study of haemosporidian parasites, and indeed, the discovery of malarial parasites themselves, would not have occurred were it not for the careful examination of avian symbionts, I begin this dissertation with a chapter on protocols and field methods for the study of avian symbionts. The aim of this chapter is to provide avian field biologists, regardless of prior experience, with the information necessary to begin collecting and preserving avian symbionts ranging from blood parasites and bacteria to ecto- and endoparasites. The chapter represents a culmination of field experience, and my personal philosophy on the importance of comprehensive sampling of the avian "ecosystem".

It was exactly such field experience collecting avian symbionts that led me to discover the fascinating world of haemosporidian parasites. Following the introduction to field and sampling methods for the study of avian symbionts, I explore several aspects of haemosporidian biology, and the remaining chapters of this dissertation examine both the ecology and evolutionary biology of malarial parasites in wild vertebrates. One major aim of this dissertation has been to expand the sampling of potential haemosporidian hosts in the Afrotropics – particularly of birds and small mammals (bats, rodents, and shrews) – to improve resolution of the haemosporidian Tree of Life. By doing so, our ability to understand patterns of host-switching and to test evolutionary hypotheses for haemosporidian parasites will be greatly improved. Improving the haemosporidian phylogeny may also shed light on the origin of *Plasmodium* parasites in primates – particularly in humans. Characterizing patterns of host–parasite associations in wild populations also allows us to produce models that can predict the probability that individuals of a particular species will be infected by specific haemosporidian parasites, which is the second major aim of this dissertation.

Beyond improving taxonomic sampling of haemosporidian parasites globally, there is a great need for improved methods for isolating and sequencing genomic data from hosts that pose unique challenges (e.g., hosts that have nucleated red blood cells, and hosts exhibiting coinfections). The significance of this goal has grown as "next generation" sequencing technologies have changed the way we analyze genomes. In particular, avian haemosporidian parasites are of great interest due to their immense diversity, universal distribution, and hypothesized position at the root of the haemosporidian phylogeny (Figure 1.4**)**. In other words, all extant malarial parasites appear to have arisen from an ancestor of avian-parasitizing origin.

Figure 1.4. Hypothesis of haemosporidian phylogeny based on the study of Lutz et al. (2016).

However, avian hosts tend to exhibit low levels of chronic malarial infection. Furthermore, birds have nucleated red blood cells. These factors have made it challenging to isolate pure parasite

DNA from avian haemosporidians, and have limited our ability to include avian parasites in comparative genomic analyses. Thus, the third aim of this dissertation is to present a new method for isolating malarial parasite DNA from intra-erythrocitic parasites of avian hosts. The ability to isolate DNA of malarial parasites from individual cells has important implications for comparative genomic analyses, which in turn have important implications for the study of the molecular evolution of malarial parasites. In the final chapter of this disseration, I combine data obtained from laser-isolated material with data provided by the Wellcome Trust Sanger Institute to explore the molecular evolution of the avian parasite, *Plasmodium relictum*. In doing so, I find a unique set of amino acid sites that may be under positive Darwinian selection. These amino acid sites are located within genes that encode proteins involved in host cell invasion and immune response evasion. Because they interact directly with the host, these genes are crucial for successful invasion and propagation of parasites within the host. Indeed, one of the genes that was identified as being under positive Darwinian selection, merozoite surface protein 1 (*msp-1*) has been considered as a target for vaccine development in the battle against human malarial parasites.

With this dissertation, I hope to contribute to several areas of haemosporidian research. Finding new ways to examine host–parasite ecology and the factors driving malarial parasite prevalence in vertebrate hosts may provide insights into the evolution of host life history traits, for example. By improving sampling of malarial parasites in African vertebrates, I have identified over 200 novel parasite lineages in birds and bats, and have used genetic data from these lineages to improve resolution of the haemosporidian Tree of Life. Finally, I have tested the applicability of laser microdissection techniques to single-cell isolation of malarial parasites in avian red blood cells, and examined the extent to which positive Darwinian selection has shaped the molecular evolution of the avian malarial parasite, *Plasmodium relictum*. Further exploration of parasite

diversity in the tropics and efforts to develop new methods for comparative genomic studies of haemosporidian parasites will continue to be important for improving our knowledge of the ecological and evolutioary history of malarial parasites. This dissertation is my contribution to what has become a rapidly growing field of research, concerning one of the most ancient diseases known to man.

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CHAPTER 2

METHODS FOR SPECIMEN-BASED STUDIES OF AVIAN SYMBIONTS[1]

Abstract

The collection of avian voucher specimens has long played an important role in the study of the basic biology, ecology, and evolution of birds. However, symbionts (such as parasites and pathogens) of avian hosts have been largely neglected by ornithologists, and are largely underrepresented in most major museum collections. Museum-oriented research expeditions to collect bird specimens capture a diversity of metadata, but the proper collection of symbionts for optimal use in downstream research projects remains uncommon. In this chapter, we provide methods for the comprehensive sampling of a diverse suite of symbionts from avian hosts, including blood parasites (hematozoans), microbial symbionts (bacteria and viruses), ectoparasites (arthropods), and endoparasites (helminths), while attempting to illustrate the research avenues opened by collecting such samples. Our objective is to encourage a view of birds as ecosystems in and of themselves, and to empower field ornithologists, particularly those participating in the collection of voucher specimens, to sample the plethora of microorganisms that live in and on avian hosts. By collecting these additional specimens, ornithologists will not only unlock new aspects of avian biology, but will also expand the scientific community's ability

[1] Lutz HL, Tkach VT, Weckstein JD. (In Press) Methods for specimen-based studies of avian symbionts. In: Michael Webster, editor. The role of collections in Ornithology: The extended specimen. Boca Raton, FL: CRC Press.

to address ecological and evolutionary questions, while aiding in the discovery of new parasitological biodiversity and maximizing the utility of the "extended" avian specimen.

Introduction

Collections of avian specimens have been used to address complex ecological and evolutionary questions, and these museum specimens have served as an invaluable resource to the scientific community for centuries. As we develop new tools and methods, the scientific potential of individual bird specimens continues to expand, demanding that we take a more comprehensive approach to collecting modern whole bird specimens – considering them as ecosystems in and of themselves. Birds are capable of hosting a plethora of symbionts, some visible to the naked eye, and others microscopic, some ectoparasitic and some internal (Figure 2.1). Relationships between these symbionts and their avian hosts range from mutualistic, to parasitic, to pathogenic, and all of these have the potential to influence avian behavior, ecology, and evolution (Atkinson et al. 2008; Combes 1996; Combes et al. 1996). Indeed, studies of avian parasites and pathogens have allowed ornithologists to address many important questions, from understanding how avian life history traits increase or decrease prevalence and probability of parasite infection (Clayton et al. 1992; Clayton and Walther 2001; Fecchio et al. 2011; Lutz et al. 2015), to tracking how avian populations have shifted their distributions over time (see other chapters in this volume). Because most birds are volant, they have also provided an important system for studying the evolution of virulence in rapidly-spreading emerging pathogens (Hochachka and Dhondt 2000), and have brought to light the importance of broadly sampling potential hosts when studying the origin of epidemics (Dhondt et al. 2014; Kilpatrick et al. 2006).

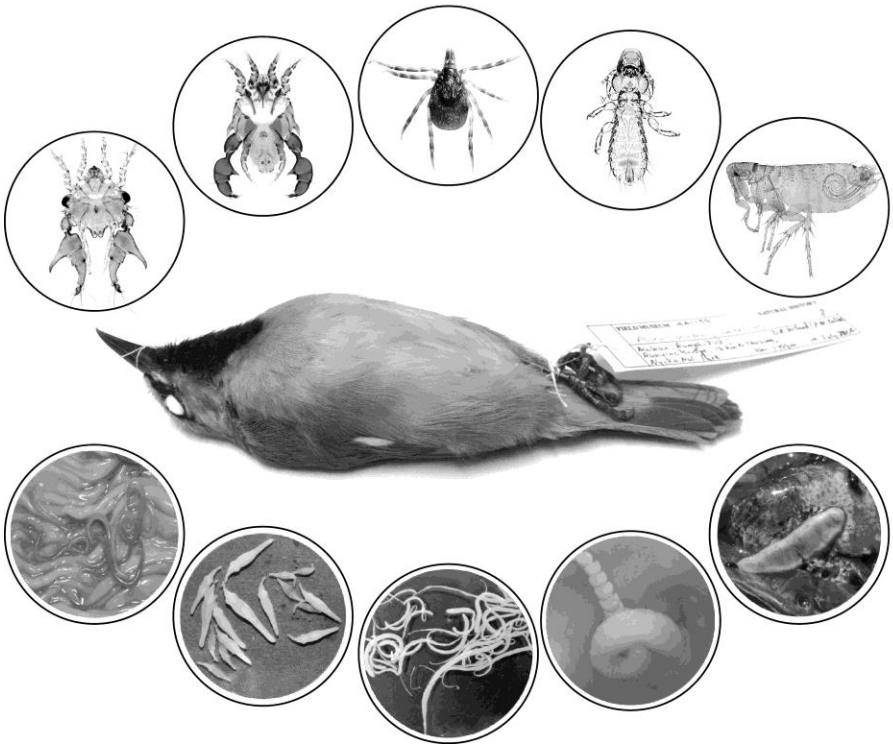

Figure 2.1. Birds can be thought of as ecosystems in and of themselves, serving as hosts for a plethora of symbionts from disparate branches of the Tree of Life

Specimen-based studies of avian symbionts are particularly useful for studying co-phylogenetic history and macroevolutionary patterns in avian hosts and parasites (Johnson and Clayton 2003a; Johnson et al. 2011; Weckstein 2004), as well as spatio-temporal relationships between birds and their environments (Galen and Witt 2014; Parker et al. 2011). For example, studies of museum specimens dating back to the early twentieth century have allowed researchers to determine when avipoxvirus was first introduced into endemic Galapagos finches and mockingbirds (Parker et al. 2011), and Hawaiian forest birds (Jarvi et al. 2007). In separate studies, the sampling of haemosporidian (malaria) parasites across a broad range of avian hosts has led to generalizations about the ability of host life history traits to predict rates of parasitism. For example, flocking behavior, nest type, and nest height or foraging stratum have been significantly linked to rates of parasitism for malarial parasites in both the Neotropics (e.g., Fecchio et al. 2011; Fecchio et al. 2013) and the Afrotropics (Lutz et al. 2015).

Haemosporidian parasites of birds have been studied for more than two centuries, and the knowledge we have acquired from these model parasites has informed the study of human and other primate malaria. The incredible diversity comprising avian haemosporidians (Valkiūnas 2005), paired with the known selective pressure these parasites impose on their hosts (Samuel et al. 2015), make them an important group to consider when studying certain aspects of avian biology. In extreme cases, avian malaria can have devastating effects when introduced to naïve populations, as occurred on the Hawaiian Islands, where several species of Hawaiian honeycreepers were driven to extinction by introduced malarial parasites (Atkinson and LaPointe 2009). Extinction caused by malaria is an extreme and rare occurrence, in fact, by most outward measures of health, infected individuals appear to suffer little from malaria (Valkiūnas 2005). However, evidence suggests that subtle, long-term fitness effects are at play in wild birds: chronic malaria has been linked to telomere degradation and senescence in great reed warblers (Asghar et al. 2015), as well as reduced quality of offspring and overall lower reproductive success of adults (Knowles et al. 2010). Such documented influences of microscopic blood parasites on avian hosts cannot be ignored when considering host ecology or evolutionary biology.

In some cases, parasites may reveal important information about the evolutionary history of their avian hosts. One of the notable attributes of ectoparasites such as avian chewing lice (Phthiraptera) is that they are permanent ectoparasites, living their entire life cycle on the host (Johnson and Clayton 2003b). Another notable attribute of this system is that the life cycle of a louse from egg to reproduction is about one month (Johnson and Clayton 2003b) and thus a single annual cycle of the avian host contains 12 louse annual cycles. As a result, generation times of the parasites are much shorter than the host generation times, and the parasites therefore

evolve at a faster rate than their hosts (Whiteman and Parker 2005), allowing ectoparasitic lice to serve as markers of recent host evolutionary history. Indeed, in some cases the DNA of ectoparasitic lice may serve as a better proxy of recent host evolutionary history than the host's own DNA. In the example that follows, we describe a specific instance where ectoparasitic lice infecting sympatric congeneric toucans in the genus *Ramphastos* can tell us a great deal about recent host evolutionary history.

Weckstein and colleagues have been collecting associated specimens of both *Ramphastos* toucans and their ectoparasites since the 1990s in an effort to understand both their cophylogenetic and cophylogeographic histories (e.g., Price and Weckstein 2005; Weckstein 2004). *Ramphastos* toucans in Amazonian Brazil include two overlapping species complexes, *Ramphastos tucanus* and *Ramphastos vitellinus*, each of which are geographically variable and form hybrid rings around the Amazon basin (Haffer 1974). At any given locality in the basin, both *R. tucanus* and *R. vitellinus* may host the ischnoceran louse species *Austrophilopterus cancellosus* (Price and Weckstein 2005; Weckstein 2004). Within the *R. vitellinus* species complex, geographic variation in coloration clearly shows a break in various Amazonian regions, including across the mouth of the Amazon river in eastern Amazonia; this break is also indicated by the subspecific taxonomy of this complex, and it is clear that there is no ongoing gene flow between the *R. vitellinus* subspecies across this riverine barrier. In contrast, *R. tucanus*, which shows east/west variation in coloration, does not exhibit a break across the mouth of the Amazon river (Haffer 1974). Instead, the eastern Amazonian subspecies *R. tucanus tucanus*, which has a reddish-orange bill, is found on both the north and south banks near the mouth of the Amazon River (Figure 2.2). Thus one is left to wonder whether the absence of north-south variation in coloration in *R. t. tucanus* across the mouth of the Amazon river is due to ongoing gene flow or,

alternatively, recent cessation of gene flow, such that there hasn't been sufficient time for divergence in plumage coloration to accrue. Genetic data from *R. tucanus,* for both mitochondrial DNA (mtDNA) and nuclear introns, shows the north and south bank that *R. tucanus* have similar or shared haplotypes, which is consistent with a history of either ongoing or recent cessation of gene flow (Weckstein 2004, unpublished data). However, upon inspection of mtDNA (cytochrome oxidase subunit I) sequences for the ischnoceran chewing louse *A. cancellosus* parasitizing these birds, a distinct genetic break across the mouth of the Amazon river is found, with louse individuals in the Guyanan shield (*A. cancellosus*[1], Figure 2.2 B) differing by uncorrected *p*-distance of 11.2% from those on the south bank of the river mouth (*A. cancellosus*[2], Figure 2.2 B). Thus, the lice are telling us that there is no ongoing dispersal of *R. t. tucanus* between the north bank and the south bank, and that any similar or shared DNA sequences between host populations on either bank of the Amazon river mouth are therefore due to retention of ancestral DNA polymorphism on account of recent cessation of gene flow (Weckstein 2004, unpublished data). This example is simply one of many that highlight the value of making detailed collections of birds and their associated parasites. One could perform similar studies using myriad parasites with different life history characteristics to reconstruct the evolutionary history and ecology of their avian hosts.

Among avian symbionts, those with parasitic life histories (on which we will focus the majority of this chapter) are particularly diverse (Windsor 1995), comprising an incredible 30%–70% of known biodiversity on our planet (de Meeûs et al. 1998; de Meeûs and Renaud 2002; Poulin 2005; Timm and Clauson 1987; Windsor 1998). For a variety of reasons, they are

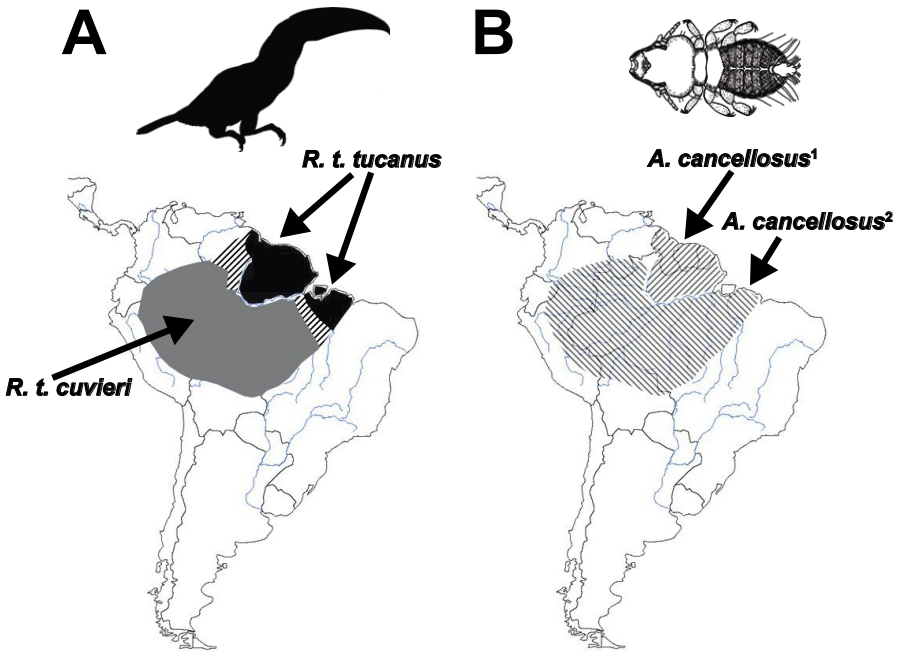

Figure 2.2. A) Map showing the distribution of *Ramphastos tucanus* subspecies in Amazonia. Gray indicates the range of *R. t. cuvieri*, black indicates the range of *R. r. tucanus*, and hash marks indicate zones of hybridization between these subspecies. B) Map showing the distributions of the divergent mtDNA lineages (Weckstein, 2004) of the toucan louse *Austrophilopterus cancellosus.*

435 important elements in the study of biodiversity (Brooks and Hoberg 2000; Brooks et al. 2001;
436 Combes 1996; Combes et al. 1996; Dobson et al. 2008; Parker et al. 2006; Whiteman and Parker
437 2005). First, parasites can have important impacts on the health, demography, behavior, and
438 evolution of their avian hosts (Combes 1996; Combes et al. 1996; Parker et al. 2006). Second,
439 parasites are ubiquitous (Combes et al. 1996) with most, if not all, birds carrying many parasite
440 species. For example, an individual bird can harbor lice, mites, ticks, hippoboscid flies, fleas,
441 spiny-headed worms, tapeworms, flukes, roundworms, and protozoans (in addition to a plethora
442 of bacterial, fungal, and viral symbionts). Third, only a fraction of the parasite species on earth
443 has been identified (Brooks and Hoberg 2001), and historically the effects of parasites on non-
444 game wild avian hosts have been understudied (Atkinson et al. 2008; Atkinson and LaPointe
445 2009; Parker et al. 2006). Lastly, many parasites can be successfully used to make inferences

about host ecology, population biology, and evolutionary history, including historical biogeography (Nieberding and Morand 2006; Nieberding and Olivieri 2007; Whiteman and Parker 2005). Thus, there is a critical need to study the birds and their symbiotic associates (parasitic or otherwise), to understand the interdependencies in the web of life, reconstruct the evolutionary history of life on our planet, and stem the tide of extinction.

Biodiversity inventories of birds and their associated symbionts are the first step towards this end, and proper methods of collection and preservation are essential for correct identification and documentation of both host and symbiont species. A great deal has been written about the importance of avian biodiversity surveys (Balmford and Gaston 1999; Gregory et al. 2003; Lawton et al. 1998; Norris and Pain 2002) and methods for obtaining, preserving, and preparing bird specimens (e.g., Johnson et al. 1984; Proctor and Lynch 1993; Winker 2000). The importance of collecting avian specimens and voucher specimens in general has also long been acknowledged (e.g., Rocha 2014; Winker 1997). However, relatively little has been written regarding how to sample these host specimens for the high diversity of symbionts living on and in them. Although a number of publications have addressed collecting specific groups of parasites and other symbionts (Clayton and Walther 1997; Dubinina 1971; Owen 2011), these publications are scattered across the scientific literature. By far the most comprehensive description of procedures aimed at a complete parasitological investigation of birds was published by Dubinina (1971). This 129-page manual includes an overview of avian anatomy and morphology, step-by-step procedures for examining the entire avian host body for parasites, and directions for proper field fixation and post-fixation protocols. However, this manual was published only in Russian, and is now difficult to obtain. Furthermore, the introduction of modern research methods and tools since the 1970s has dramatically changed requirements for

specimen fixation and preservation, leaving many of the methods presented in the aforementioned work outdated.

Therefore, we outline here the general workflow, methods, and standards for comprehensive sampling and proper preservation of avian symbionts that we consider to be optimal for a variety of modern and traditional downstream biological research applications. Our goal is to optimize knowledge about each avian host and its symbionts, collected and prepared as traditional museum specimens, by broadly sampling four major symbiont categories: blood parasites (hematozoa), microbial symbionts (bacteria and viruses), ectoparasites (arthropods), and endoparasites (helminths). The structure of this chapter reflects the order in which samples from these major groups are generally collected in the field workflow. We will not discuss the details of avian specimen preparation methods, as we assume that readers are already familiar with standard avian museum specimen preparation and data collection. If not, then the reader can refer to Winker (2000) or other papers cited above, which provide an overview of methods for preparation of bird specimens and the typical data fields that are recorded for each avian specimen. Following the final section on detailed protocols for symbiont sampling and preservation, we summarize a basic sampling workflow that can be applied in most field situations. We hope that this chapter provides a useful resource for avian collectors and field researchers, helping us edge closer to a more complete sampling of each avian "ecosystem."

Before you begin: the field notebook

As with host specimens, careful field notes help to capture valuable metadata during avian parasite collection events. We use 100% cotton fiber, acid free archival paper with

preprinted data fields and use archival ink (e.g., Pigma) to write notes on these "parasite field notebook" pages, which complement the host catalog notebook pages (Figure 2.3 A–B). In these field notes, we record basic data such as host species sampled, locality, date sampled, parasite collector doing the sampling, and whether or not anything was found (it is important to note when no parasites are found, so as not to be mistaken for a lack of sampling effort). The notebook pages use a series of checkboxes to denote what sampling was completed and leave room to describe what was collected from a given host specimen. One of the most critical data fields in this parasite field notebook is the host field number, which is used to link parasite samples (e.g., vials of ectoparasites, blood smears, etc.) to a host voucher specimen. However, for this unique identifier to be useful for parasite sampling, it must be assigned to the host specimen *before* the first parasite sampling begins, and thus before a field preparation number is usually assigned (often a personal catalog number assigned by the host specimen preparator upon entry into the personal field catalog). In our experience, there are multiple ways that this can be handled. One is to immediately assign a tissue or parasite number to each host specimen. Many museums use a separate tissue catalog to track the condition and handling of tissue samples collected in the field and this number is noted on the voucher data label and host field catalogs. Another option is to assign either a special parasite field number or general host catalog number that follows the host specimen through all steps of sampling, and also note this number on the host field label, host field notebooks, and on a ~4 x 6" host field sheet that follows the host specimen through parasite sampling and preparation (Figure 2.3 C). This host field sheet is a convenient way to maintain notes on the sampling steps that have been performed, as well as to note host data (e.g., weight, soft-part colors, etc.) before it is written in the catalog. We typically modify these sheets prior to expeditions to include a country acronym for collection numbers

(e.g., “UGA” for Uganda), the year, and fields for specific tissues or samples we may be collecting for various projects.

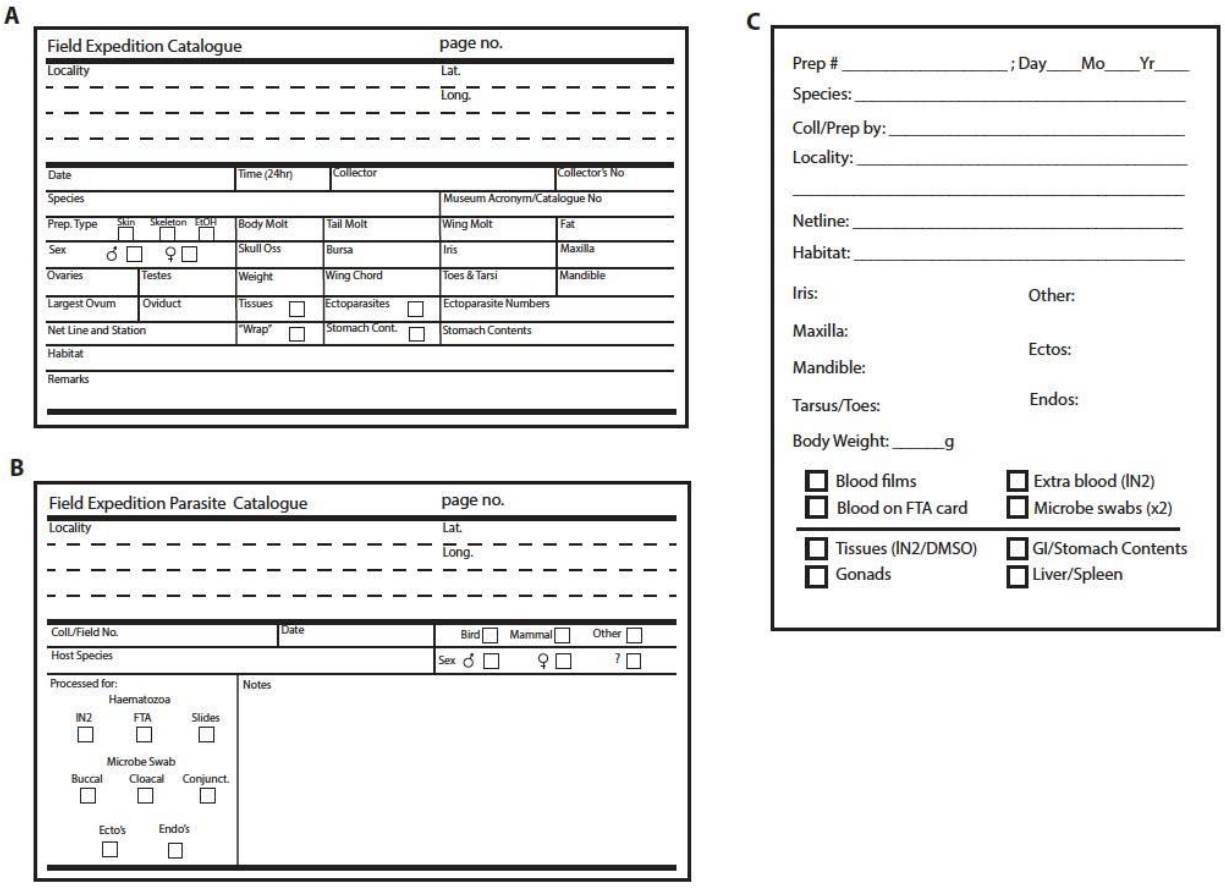

Figure 2.3. The Field Notebook. (A) Standard host catalog fields. (B) Example of parasite catalog fields. (C) Field sheet to be kept with host specimen as it goes through various stages of sampling and processing.

Sampling protocols for the study of blood parasites (haematozoa)

As with other taxonomic groups, the systematic study of avian haematozoans depends on both morphological and molecular data – both of which have their advantages and disadvantages. Phenotypic traits of haematozoan parasites may be convergent (Martinsen et al., 2008) and can

be highly plastic depending on the host and the conditions during processing of blood smears (Valkiūnas 2005). Furthermore, haematozoan parasitemia is generally quite low in birds, which can lead to improper diagnosis of infection by microscopic analysis (Richard et al. 2002). The development of molecular protocols has provided more reliable diagnostic methods, and has led to the discovery of hundreds of novel haematozoan parasite lineages (Bensch et al. 2009). In addition to improving detection capabilities, molecular methods and the development of phylogenetic markers are proving increasingly useful for studying evolutionary relationships in the haematozoan Tree of Life (Perkins and Schall 2002; Perkins 2014; Martinsen et al. 2008). However, molecular data are prone to error in cases of multi-species infections, and, alone, are insufficient for the taxonomic description of novel parasites. Therefore, when sampling birds for haematozoan parasites, it is important to collect blood for both morphological and molecular analyses.

Blood collection and storage

For live birds, blood can be drawn immediately after recording soft-part colors (maxilla, mandible, nares, eye-ring, tibiotarsus, and feet). This can be done alone, or with the help of a partner, taking experience and the size and vigor of the bird into consideration. The top priorities at this point should be proper handling of the live animal to reduce stress and suffering, and rapid processing of the blood sample once it has been drawn.

Blood from live birds can be obtained from several parts of the body, including the femoral artery, the brachial/ulnar vein, a clipped toenail, or in the case of shot or otherwise dead birds, directly from the wounds, body cavity, or heart. Although blood from a dead bird will still

provide useful material for molecular analysis of some parasites, fresh blood is desirable for haemosporidian studies, due to morphological changes elicited in these parasites by a drop in temperature and/or exposure to air. We have found brachial and jugular venipuncture to be the most efficient in both small and large birds – these veins are easily visible, and in most cases can be sampled by one person working alone. Sampling blood by clipping the toenail should be avoided, as it frequently leads to the introduction of debris into the sample (if not cleaned properly), produces a relatively low volume of blood, and may be quite painful for the animal (the toenail clipping method is not approved for most species by The Ornithological Council, Washington, DC)(Gaunt and Oring 1999).

For the majority of bird species, a small gauge needle (22–27 ga) is best for sampling blood. Smaller gauge needles reduce the likelihood of hematoma, but may increase the probability of hemolysis, affecting downstream hematocrit measurements and blood smear quality. We typically use 25–27 gauge needles. Be sure that your needles are designed specifically for subcutaneous use (frequently denoted "SubQ"), as other needle types (e.g., the ones made for intradermal use) are blunt-tipped and inappropriate for venipuncture. Before searching for the vein, it is helpful to wet the area with alcohol or water to clear the feathers out of the way, which makes the vein more visible. Some researchers prefer to use vaseline, which holds the feathers out of the way and causes the blood to bead up more effectively, making it easier to be drawn neatly into a capillary tube. We avoid the use of vaseline due to its matting affect on the feathers of birds that are to be preserved as museum vouchers. The needle should be placed parallel to the vein, bevel side up. With very light pressure, insert the needle ~0.5–1 mm into the vein and remove quickly. A small drop of blood will then form, and can be collected directly into a heparinized capillary tube. Do not place the capillary tube directly against the

vein, as this can inhibit blood flow. Likewise, hyperextension of the wing or leg from which blood is being drawn can restrict blood flow. A typical microhematocrit capillary tube holds about 0.075 ml (75 µl) of blood. The volume of blood collected will depend on the size and condition of the bird, but for birds that are to be collected as specimens, 1–2 hematocrit tubes (0.075 – 0.15 ml) is more than sufficient for molecular and morphological analyses of haematozoan parasites (we often rely on < 0.05 ml of blood for our studies). If the bird is to be released rather than collected, be sure to take no more than the equivalent of 1% of the bird's body mass in volume of blood (Fair et al. 2010). After taking the blood sample (and performing other relevant tasks), check to make sure that the bird is in good condition, and that it is alert with eyes open before releasing it.

Once drawn, blood should be stored for both microscopic and molecular analyses. Blood smears for microscopic analysis of parasite morphology should be prepared immediately after drawing blood (see next section). At this point, it is helpful to have a partner to whom you can hand the bird for euthanization. Alternatively, one person can bleed the bird and a second person can make the blood smears. The person taking the blood sample can immediately euthanize the avian specimen after blood has been collected. See The Ornithological Council's Guidlines (www.naturalhistory.si.edu/BIRDNET/, August 20, 2014) for information on appropriate methods for euthanizing birds for preparation as museum specimens. Following the preparation of blood smears, multiple methods may be used for preserving whole blood for DNA studies: flash freezing blood in liquid nitrogen (the "gold standard"), storage of blood on Whatman® FTA® Classic Cards, and storage of blood in a DNA preserving buffer (e.g., Queen's lysis buffer, 95% ethanol, etc.). We typically place a small amount of blood on an FTA card for quick access in the lab, and store the remainder in liquid nitrogen for long-term storage in a cryogenic

facility. Many researchers prefer to use 95% ethanol for the storage of blood for molecular analysis, as it is inexpensive and easily accessible in remote locations. Blood samples on FTA cards (or other filter paper) should be stored in a dry space free of contamination, such as a ziploc bag with silica beads. FTA cards come with pre-printed subsections for applying samples. Because we only require a small amount of blood for molecular analyses, we typically subdivide the cards using a custom-made stamp so that more samples may be stored on an individual card (e.g., we store nine unique blood samples instead of four).

It is important to note that unnecessary handling of birds can lead to a loss of ectoparasites, such as hippoboscid flies, which are volant and may leave the host when they sense a disturbance. Thus, it is best to quickly euthanize the avian host specimen, swab it for microbial symbionts (see following section), and then isolate the carcass in a ziploc (or similar) bag containing a fumigant for disabling associated ectoparasites. With this system, blood samples are rapidly prepared and the avian host is quickly relieved of suffering. It is very important to label both the host and blood samples (slides, vials, FTA cards, etc.) with a unique identifier (e.g., host field number or tissue number) before proceeding. This is particularly true if a large number of birds are in queue to be processed, or when multiple researchers are processing the avian host for different parasites and pathogens. Regardless of the circumstances, it is generally good practice to label specimen tubes immediately after sampling, and to tie a leg tag with this unique identifier directly onto the avian host immediately after it is euthanized.

Preparation and fixation of blood smears

The quick preparation of blood smears is important for two reasons. First, the temperature change of blood can have profound effects on haemosporidian parasite morphology, making subsequent analyses of blood parasites complicated, or even impossible. This may be linked to the life history of the parasite, with the temperature change simulating transfer of the parasite from the vertebrate to the invertebrate host, and inducing the progression of the parasite into the next stage of its life cycle (Valkiūnas 2005). Second, even when collected in heparinized microhematocrit tubes, blood can begin to clot, particularly in hot environments. If you are working alone and experience some delay before processing blood, it is helpful to first dab the end of the microhematocrit tube onto the FTA card (or other sterile paper, if planning to store blood in lysis buffer) before applying a blood drop to the glass slides for preparation of blood smears. This removes any blood that has clotted at the end of the microhematocrit tube, allowing blood to flow more freely from the tube. As has been described in many useful guides (e.g., Gilles 1993; Owen 2011; Valkiūnas 2005), smears should be prepared on clean glass slides. Dust particles, grease, and scratches will significantly decrease the quality of your blood smear, and contaminated slides should be avoided. Unused slides that have been contaminated by dust or debris can be cleaned using ethanol and kimwipes if necessary. A small drop of blood no larger than 3 mm in diameter is all that is needed to produce a good blood smear. A common error in the preparation of blood smears is the use of too much blood. The drop should be placed at one end of the slide, and a second clean "smearing" slide backed up at a 30˚–45˚ angle until it is touching the drop (Figure 2.4 A–B), at which point the blood will spread across the back end of the smearing slide via capillary action. The smearing slide should then be pushed forward, briskly and smoothly, with blood trailing behind it (Figure 2.4 C). If done properly, the blood smear should be in the shape of a bullet, with the densest concentration of the blood near the

origin of the drop, and the edges of the smear feathering out towards the end (Figure 2.4 D). It is important to produce multiple slides per individual when possible. Not only does this increase the number of fields that can be searched for haemosporidian and other blood parasites, but more importantly, it will allow for the deposition of slides at different institutions (which is often important when operating with collaborators). In the interest of maximizing the number of searchable fields, while maintaining the ability to share slides with different institutions, it is

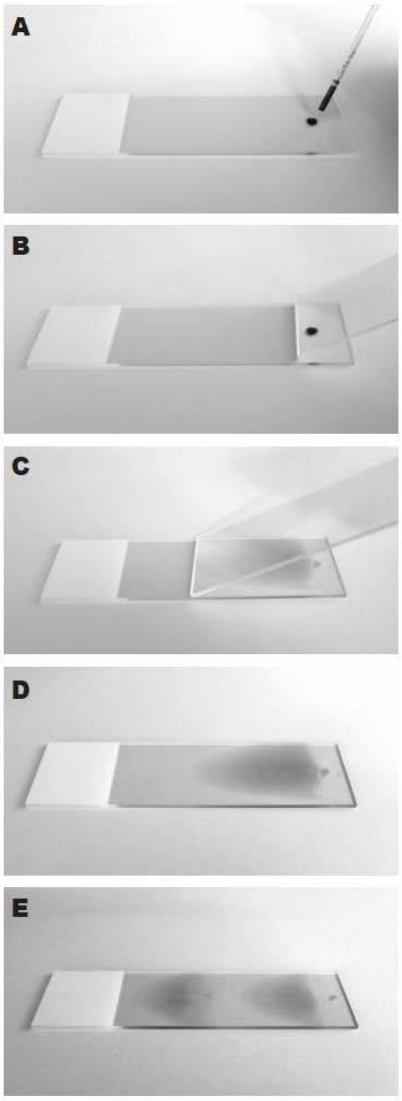

Figure 2.4. How to make a blood smear. (A) Place small drop of blood from microhematocrit tube near end of slide. (B) Back second slide up to drop of blood at 30˚–45˚ angle. (C) Move the "smearing" slide quickly and smoothly across to spread the blood in a thin film. (D) Single thin blood smear. (E) Example of two blood smears prepared on a single slide.

quite practical to produce multiple blood smears from the same individual on individual glass slides (Figure 2.4 E). The ability to do this will vary with level of skill, environmental conditions, and the condition of the bird.

It is best to fix blood smears as soon as possible once they are air-dried, placing them in 100% methanol for one minute. If methanol is unavailable, it can be substituted with 96% ethanol and an extended fixation time of three minutes. Slides can be placed back to back in a coplin jar containing fresh methanol. Replace the alcohol frequently (every 2–3 batches of slides) to limit the effects of dilution and debris. Allow slides to air-dry face up, and once dry, place the slides back to back in a plastic slide box for storage. Alternatively, slides can be individually wrapped with paper, such as a kimwipe, and bound together using rubber bands. Store fixed blood smears with silica beads, and stain as soon as possible. Although most staining agents containing methyl-blue will allow for microscopic detection of hematozoa, Giemsa remains the "gold standard", and is the most commonly used stain for parasitological studies of haematozoan blood parasites. Rapid staining methods used for diagnosis of human malaria (e.g., Field's or Romanowsky stains) are less stable than Giemsa and prone to fading, and therefore are not appropriate for long-term storage and taxonomic studies. It is best to purchase a high quality Giemsa stain and produce your own staining buffers. Staining formulas and protocols are described in Appendix A.

In many field situations, it is not possible to stain slides on the same day, or even within one week, of preparation (which is recommended). Older blood smears, if made and fixed properly in the field, are still useful for taxonomic research. However, it is a good idea to "re-fix" the slides once back in the laboratory by dipping them again in 100% methanol for one minute,

and allowing them to air-dry (R. Barraclough, personal communication, June 2014). Older slides tend to absorb more stain, so Giemsa staining concentration and staining time should be reduced. It is a good idea to test your staining protocol on one slide before processing an entire batch. If the blood smear is overly dark and blue, simply dilute your stain or reduce the amount of staining time (add more stain or time if the slide appears too light). Once staining is complete, slides should be placed in a durable slide box and kept in a cool, dry environment for long-term storage. These slides will serve as vouchers, and can be referred to at any point for morphological analyses of myriad haematozoan parasites found in avian hosts (Figure 2.5).

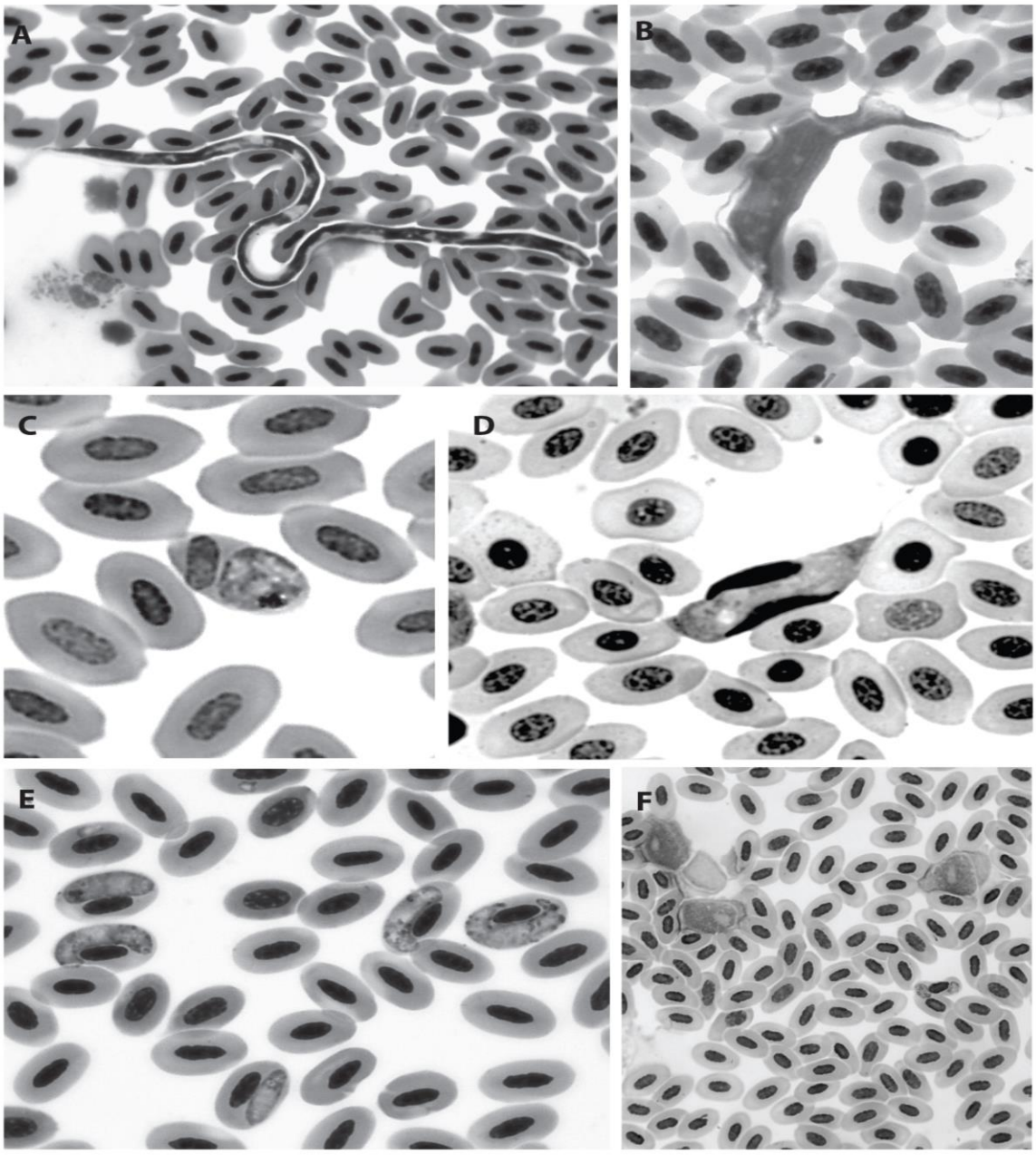

Figure 2.5. Microphotographs from Giemsa-stained blood smears of haematozoan parasites found in birds. (A) Microfilarial nematode ex *Pycnonotus barbatus*, Vwaza Marsh Wildlife Reserve, Malawi (B) *Trypanosoma* sp. ex *Pycnonotus barbatus*, Vwaza Marsh Wildlife Reserve, Malawi (C) *Plasmodium* sp. ex North American passerine (D) *Leucocytozoon toddi* ex *Meleagris gallopavo*, Ithaca, NY, USA. (E) *Haemoproteus* sp. ex *Ispidina picta*, Vwaza Marsh Wildlife Reserve, Malawi (F) Co-infection with *Leucocytozoon* sp. and *Haemproteus zosteropis* ex *Zosterops senegalensis*, Nyika National Park, Malawi.

674

Sampling protocols for the study of microbial symbionts (bacteria, fungi, and viruses)

Studies of microbial symbionts in wildlife are in their relative infancy, and methods for sampling bacterial, fungal, and viral symbionts of birds are still being developed and improved. As our understanding of the interplay between microbial symbionts and avian evolution and ecology grows, so too should collections of samples from vouchered birds that are appropriate for studying these microbes (e.g., gastrointestinal tracts, fecal, buccal, and conjuctival swabs, etc.). The collection of such samples will ultimately provide important time series for the study of changes in microbial diversity in birds, which may allow researchers to measure the effects of environmental phenomena such as climate change, anthropogenic habitat disturbance, as well as the impacts of naturally occurring phenomena, such as dispersal and colonization, epidemics, and naturally fluctuating food cycles. Avian gut microbiota have probably received the greatest amount of attention (for a review of the current trends in this area of study, see Waite and Taylor 2015), and recent studies (e.g., Hird et al. 2015; van Dongen et al. 2013) provide useful methodological descriptions for studying the avian gastrointestinal microbiome. However, microbial symbionts are by no means restricted to the gastrointestinal tract, and other areas to consider when sampling a bird for microbial symbionts include the respiratory tract, conjuctiva, nares, and feathers.

As microbiome studies are still in their relative infancy, and best methods and practices are still being developed, we encourage researchers to check the most recent literature for proper sampling and storage techniques. Methods of preservation for the study of viruses, in particular,

vary substantially, and knowledge of the viral family of interest is important when determining sample preservation methods. Because this area of study is in a state of rapid development, we will provide here only the most basic advice on when and how to incorporate sampling for microbial symbionts into the field workflow, based on our experience.

Sampling for microbial symbionts (bacteria, fungi, viruses) should be conducted immediately after euthenization. Following euthanization, insert separate cotton-tipped sterile applicators into the a) cloaca, b) buccal cavity, and c) conjuctiva, and rotate several times within each region to swab the area as thoroughly as possible. The applicator can then be placed into a sterile collection tube, the handle broken off, and the tube sealed. Proper storage of swabs will depend on the questions being addressed and resources available. Some options include immediate storage in liquid nitrogen, RNAlater, or other buffers, (see Vo and Jedlicka 2014 for examples on downstream processing methods). We strongly encourage researchers to consider incorporating these simple and relatively inexpensive methods into their sampling regime, as few collections of microbial symbionts from wild birds currently exist, and the benefits and impacts of longitudinal studies are as yet undeteremined.

Sampling protocols for the study of ectoparasites

Birds are parasitized by a wide variety of ectoparasitic arthropods including, but not limited to fleas, hippoboscid flies, lice, mites, and ticks (see Clayton and Walther 1997) (Figure 2.6). Here, we will focus on methods for sampling these parasites from dead avian host specimens collected during biotic survey field expeditions, although there are a variety of additional methods that can be used to collect ectoparasites from live birds and that can be used

721 in laboratory settings (Clayton and Walther 1997; Clayton and Drown, 2001; Walther and
722 Clayton 1997). Clayton and Walther (1997) include a broad review of methods for quantification
723 and collection of avian ectoparasites.
724
725 *Fumigation and collection of ectoparasites*
726
727 The first rule for collecting ectoparasites from avian host specimens is to not allow dead
728 host specimens to come into physical contact with one another. Each freshly killed or caught bird
729 should be isolated in a separate clean bag. Birds that are mistnetted can be carried back to the
730 specimen preparation area alive using clean cloth bags. Bags should be washed thoroughly
731 between uses to avoid potential contamination of parasites between individual birds. Birds that
732 are shot can be placed immediately into a ziploc bag with a note indicating soft-part colors
733 (which may fade very quickly) and a cotton ball with a few drops of ethyl acetate on it. This will
734 begin fumigation, allowing the bird to be immediately ruffled for ectoparasites on arrival in the
735 field camp. Upon arrival into camp, each dead bird specimen in a bag should have a field number
736 assigned to its field sheet (e.g., Figure 2.3 C) so that the remaining data collected from the bird
737 (host and parasite data) can be linked to the voucher host specimen.
738

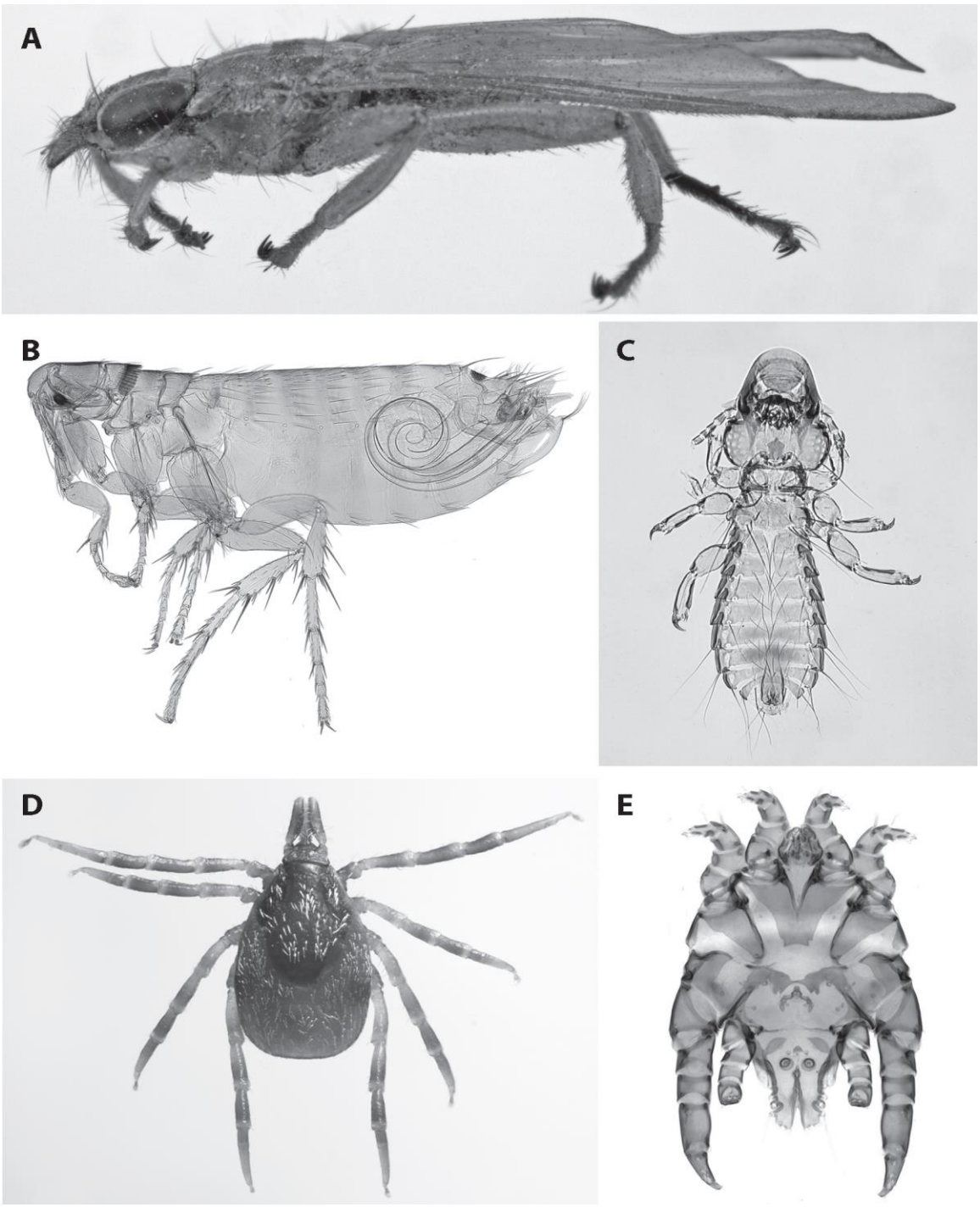

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Figure 2.6. Representive images of common ectoparasite groups found on birds. (A) Hippoboscide fly: *Icosta Americana* ex Accipiter cooperi (photo by Jason Weintraub). (B) Flea: Ceratophyllus *altus ex Campephilus magellanicus* (photo by Michael W. Hastriter). (C) Chewing Louse: Cotingacola lutzae ex Laniocera hyppopyrra (photo by Michel P. Valim). (D) Tick: *Ixodes brunneus* (photo by Lorenza Beati). (E) Feather mite: *Anomothrix machadoi* ex *Buceros* leadbeateri (photo by Fabio Akashi Hernandes).

Birds that are caught live will be euthanized after blood samples are collected. These birds should also be placed in clean ziploc bag with a cotton ball soaked with a few drops of ethyl acetate and a field sheet indicating the field number assigned to that specimen. Ethyl acetate is considered harmless to humans and yet is effective for killing ectoparasites (Fowler 1984).

After fumigating the bird for 15–20 minutes, carefully remove the bird from the ziploc bag over a large sheet of clean white paper. In the field, we typically use a lunch tray covered with a large sheet made by taping two pieces of 8.5" x 11" white paper together. In windy conditions it is a good idea to use a cardboard box or other windbreaks to block the wind. Also, one can tape the paper to the lunch tray to keep the wind from blowing the sheet away, which could result in the loss of ectoparasites. Before ruffling the bird's feathers to dislodge and remove ectoparasites, always check the inside of the ziploc bag for parasites. If any ectoparasites have fallen off of the host inside the bag, use a paint brush wetted with absolute ethanol to pick them up and place them in a new vial filled with absolute ethanol (do not use denatured ethanol). Hold the bird with one hand and use the other hand and fingers to ruffle all of the bird's feather tracts. Start with the wings, including the primaries and coverts, and then while holding the legs with one hand you can ruffle the feathers of the belly, back, and head. Then hold the head and or the beak (if the bird has a large bill) and "beat" the bird to loosen attached ectoparasites. For small birds, one can also hold them between two cupped hands and shake them up and down like dice. This also helps to loosen strongly attached ectoparasites. Furthermore, ectoparasites such as feather lice (Phthiraptera: Ischnocera) can include four ecomorphs that are specialized in different regions of the avian host's body: head, wing, body, and generalist ecomorphs (Johnson et al. 2012). Be sure to cover all of the body carefully to thoroughly sample these different ectoparasites.

Pick up all ectoparasites that fall off of the host onto the paper, using the tip of a fine paint brush moistened with absolute ethanol. Place these parasites into a vial of absolute ethanol. It is best not to use forceps to pick up ectoparasites, because this may damage morphological features on the specimens. Although many previous papers have suggested using 70% ethanol for preservation of ectoparasites (e.g., Clayton and Walther 1997), we have found that absolute ethanol is best because it preserves both the morphology and the DNA of the specimen; specimens stored in 70% ethanol will very quickly be useless for DNA extraction. However, if absolute ethanol is unavailable, 95% ethanol can be used in its place for collection and storage of ectoparasites. Place a label made with archival acid free cotton fiber paper and written using an indelible Pigma Micron pen inside the vial. The label should contain host taxon name, field collecting number, date of collection, collecting locality, and name of parasite collector. Be sure to note information on the ectoparasite collecting event in the field notes catalog. It is also important to note negative collecting events, as these data will allow one to calculate prevalence of parasites. After picking up the parasites, continue with several more bouts of ruffling until no parasites fall off the host. Before moving on to the next host specimen, clean off the collecting surface and inspect your hands to be sure there are no contaminant ectoparasites on either of them.

The ethyl acetate fumigation with post-mortem ruffling method outlined above will collect most lice, ticks, fleas, hippoboscid flies and external mites (Figure 2.6). However, this method isn't quantitative for all of these parasites. For permanent ectoparasites, such as lice, which live their entire life cycle on the host, this post-mortem ruffling method is quantitative only when conducted to a point of diminishing returns (Clayton and Drown 2001; Walther and Clayton 1997). Moreover, this method is not suitable for quantitatively sampling ectoparasites

that live inside the throat pouch, nasal cavities, feather quills, and under the skin. To thoroughly sample avian feather mites, one should visually search through the plumage using a stereomicroscope (although ruffling will allow the collection of some mites). This also allows one to note the locations where each mite taxon is found. For the subset of feather mites that inhabit the wings, one can hold the flight feathers up to the light and look for mites inserted between the feather barbs. One can then use the handle of the paintbrush to disturb and "unzip" the barbs of these feathers so that the mites fall onto the collecting paper. Other mites, such as nasal mites, require flushing the nares with water into a gallon jar, then pouring through a #200 sieve to filter out the mites. Other quantitative methods are available, such as body washing, which removes an even larger fraction of ectoparasites than post-mortem ruffling (Clayton and Drown 2001). However, this method is not practical for field survey situations, but is useful for smaller scale studies when specimens can be processed in the lab (e.g., Koop and Clayton 2013). Sometimes embedded ticks do not fall off the host after fumigation. In this case, use a forceps to grab the tick as close to the skin as possible to dislodge it without damaging its mouthparts.

After ruffling, the ectoparasite collecter will pass the host specimen on to a bird skinner who will prepare the bird specimen and gently necropsy the carcass to gather standard internal organ data. The bird skinner will then pass the carcass to the endoparasite collector for further dissection and and collection of endoparasites. The bird skinner can either sample liver and heart tissues at this time or can pass labeled tubes to the endoparasitologist to collect these tissues.

Preparation and curation of ectoparasite specimens

After returning from the field, individual vials of ectoparasite specimens can be examined to determine and quantify contents. We examine specimens in a glass dish filled with absolute ethanol and use a stereomicroscope to observe specimens and manipulate them with a paintbrush and/or bent syringe needle. Sometimes we use a glass bulb pipet to return specimens to the original vial. Always be sure that a pipet is clean before reusing it. Specimen preparation methods for each ectoparasite group are taxon-specific and can be used to produce slides for morphological examination and for slides of voucher specimens, from which DNA has been extracted. For morphological examination and vouchering for molecular projects, lice are mounted in Canada balsam using a clearing and slide mounting technique described by Palma (1978), whereas mites are mounted in Hoyer's medium (Baker and Wharton 1952). For DNA extraction of lice, we typically use a sterilized syringe needle to make a cut between the head and the thorax or between the thorax and the abdomen of the louse (depending on the taxon) (Valim and Weckstein 2011) and then place this specimen into the digestion buffer provided in the Qiagen QIAamp DNA Micro Kit (Item # 56304). We then allow the louse to be digested over two nights and then follow the manufacturer's directions. In pipetting the liquid from the digestion to the Qiagen filter, we are careful to leave the louse in the original digestion tube. We then add 70% ethanol to the tube to preserve the louse until we begin the slide mounting process. Depending on the size of the louse we elute the DNA off the filter with 50–100 µl of buffer AE. We give each louse a unique identifier that includes abbreviations for the louse' taxonomic name, host alpha taxonomic name, the date of extraction, and the tube number in that batch of extractions. Rather than wait a long time after DNA extraction, it is best to start clearing and slide mounting vouchers as soon as possible. Other ectoparasites, such as hippoboscid flies can be kept in ethanol, pinned, or slide mounted in balsam depending on the size of the fly.

831

832 ***Sampling protocols for the study of endoparasites***

833

834 Birds can harbor an astounding diversity of parasitic worms from all major groups of
835 helminths (except monogeneans), namely the cestodes (Eucestoda), digeneans (Digenea),
836 nematodes (Nematoda) and acathocephalans (Acanthocephala) (Figure 2.7). Birds can be
837 parasitized by both adult and larval stages of various parasitic worms, although birds in general
838 have fewer larval stages of helminths in comparison with other major vertebrate groups because
839 they rarely serve as intermediate or paratenic hosts of helminths. Helminths can be found in
840 virtually every part of the bird body. Although the majority are parasitic in the gastrointestinal
841 tract (GIT) – including somewhat unusual sites such as cloaca, inside of the crop, or under the
842 lining of the gizzard – many parasitize other organs, such as the liver and gall bladder, kidneys,
843 bursa of Fabricius, trachea, eyes and mouth cavity (Atkinson et al. 2008). Adult filarial
844 nematodes can be found in the body cavity, under the skin, on the brain, on the heart, and inside
845 the bones. Finally, blood flukes and larval stages of filarial nematodes (microfilariae) may reside
846 in both the venous and arterial sides of the circulatory system. Thus, a complete helminthological
847 examination of an avian host can be a very time-consuming process, especially when it involves
848 larger birds.

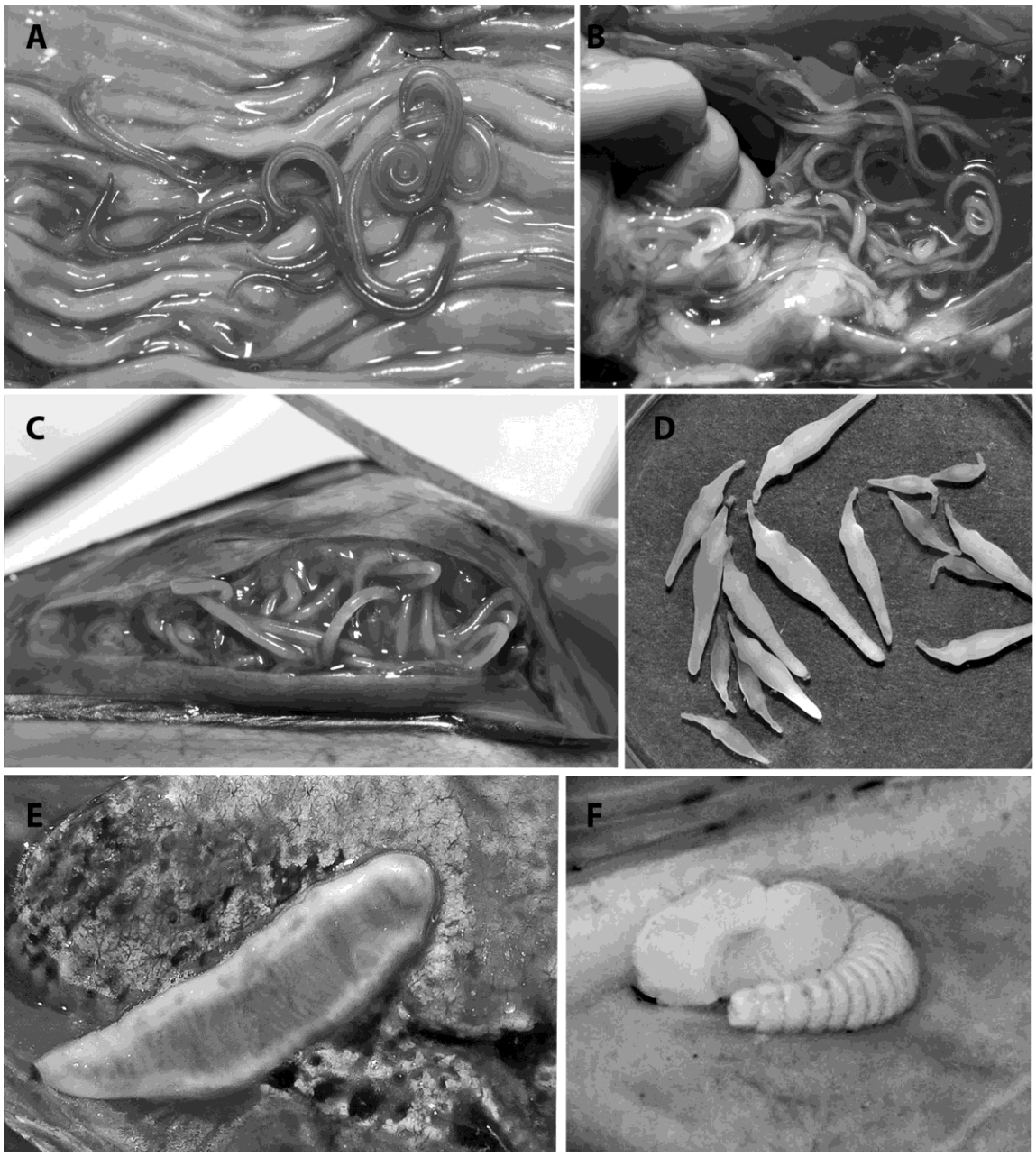

Figure 2.7. Examples of different groups of helminths inhabiting various sites in bird bodies. (A) Spirurud nematodes in stomach of anhinga *Anhinga anhinga*. (B) Filariid nematodes in body cavity of barn swallow *Hirundo rustica*. (C) Dracunculid nematode *Avioserpens* sp. under skin of the chin area of little egret *Egretta garzetta*. (D) Acanthocephalans obtained from Northern Shoveler *Anas clypeata*. (E) Hymenolepidid cestode Cloacotaenia megalops attached to the wall of cloaca of Northern Shoveler *A. clypeata*. (F) Cyclocoeliid digenean in body cavity (on the lung) of Eurasian coot *Fulica atra*.

849

It is extremely difficult to write a unified procedure for all birds due to the great diversity of avian host size, anatomical peculiarities, parasite localization, and parasite loads. For example, waterfowl and other aquatic birds usually – although not always – host greater diversity of parasitic worms that terrestrial birds. The dissection protocol below focuses primarily on helminth recovery from GIT and associated organs, where the majority of parasitic worms are expected to be found. Illustrations and descriptions of bird anatomy can be found in any general ornithology or vertebrate anatomy textbook, and are also readily available through numerous online resources.

Examination of gastroingestinal tract and other organs for endoparasites

Birds are most commonly examined for endoparasites as a part of broader ornithological studies that involve the collection of voucher specimens (e.g., study skins and skeletons) for deposition in museum collections. In such cases, a parasitologist typically receives the body or organs of an already euthanized and skinned bird, which they may proceed to dissect.

Dissection techniques may vary. When the host body is received, you should first carefully examine the exterior of the carcass (particularly around the neck) for the presence of filariid nematodes, which should be easily visible. Each avian carcass should be placed in a dissecting tray of appropriate size. In a comprehensive sampling protocol, such as the one recommended in this chapter, an incision should already have been made at this point in order to access the body cavity for tissue sampling and sexing of the host. However, if the body has not already been opened by the host voucher preparator (e.g., in cases where birds have been donated by hunters), then you will need to open the carcass by making an incision on the abdominal side

of the body. The incision should be made with scalpel or scissors along the midline of the body on its abdominal side, approximately from the level of the sternum to the level close to the cloaca, but not reaching the cloaca. When not preserving the skeleton, one can cut through the rib cage to provide easier access to the organs located in the upper part of the body (esophagus, trachea, heart, air sacs, etc.). While making any cuts be careful to not cut the GIT, as this will cause gut contents (and possibly helminths) to spill into the body cavity. This may also happen if a bird was shot and the GIT is damaged. As with the exterior of the carcass, you should inspect the interior of the body cavity for visible filariid nematodes once an incision has been made. If blood flukes (Schistosomatidae) are among the targeted parasites, use citrated saline solution (which can be prepared by dissolving 5 g of non-iodized table salt and 3 g of sodium citrate $C_6H_5Na_3O_7$ in 1L of water throughout this procedure. Make sure to pour some citrated saline into the bird body cavity as soon as you open it. If blood flukes are not a target, use regular saline throughout. Any helminths discovered should to be kept alive in saline until fixation.

Remove the complete GIT by carefully cutting the connective tissues holding it in place. Then cut the mesenteries that hold the intestinal coils together. When separating the intestine from the liver do not cut the gall bladder. Cut through the skin surrounding the cloaca to keep it intact. In young birds, the bursa of Fabricius may be found on the side of the cloaca. It is best to keep the cloaca and the bursa of Fabricius together until examination. Move the entire GIT into a tray of appropriate size (e.g., a large glass petri dish for small Passerine birds or a large glass baking dish for ducks or other large bodied birds). At this point, different parts of GIT (esophagus, stomach, small intestine, ceca, rectum, cloaca + bursa of Fabricius, etc) can be separated for subsequent examination. Remove the liver and put it in a separate dish with saline. Spleen and pancreas very rarely contain parasites, although digenean infections can be

encountered in these organs. Carefully remove the kidneys. This can be done by pulling one end of a kidney upward using forceps of appropriate size and cutting underneath with scissors. Place kidneys into a separate dish with saline. Remove the trachea and place it in a dish with saline. The trachea very rarely serves as site for helminths, but large digeneans such as *Orchipedum* in cranes and pathogenic nematodes *Syngamus trachea* in galliformes can be found there.

Disrupt air sacs with gloved hand or using scissors. Carefully rinse the entire body cavity with citrated or regular saline, and pour it from the body cavity into the pan, and then from the pan into a beaker of appropriate size. Allow the contents to settle. This process, called sedimentation, allows endoparasites and other solids to settle to the bottom and the bloody mixture in the supernatant to be discarded so that parasites may be observed and collected for fixation. Once sedimentation is mostly completed, discard the supernatant into another container by carefully pouring it off. Be sure to pour the supernatant slowly to avoid loss of the materials at the bottom of the beaker, then add fresh saline to the sediment. Shake or stir. Repeat the procedure until the supernatant is reasonably clear. Pour small portions of sediment into a petri dish and examine under stereo microscope. While some digeneans can be large, such as members of the Cyclocoeliidae, others, such as those that fall out of damaged intestine or kidneys, may be much smaller. Blood flukes or their fragments, for example, may be extremely small and transparent. Helminths should be transferred using pipettes with orifices of different sizes or lifted with curved tweezers, curved needles, or similar instruments. It is important to avoid grabbing and holding any helminths using tweezers/forceps, with exception of large nematodes and acanthocephalans, which can be taken and transferred using soft forceps. Handling helminths with tweezers almost invariably leads to their damage or destruction.

Examination of the intestine usually takes longer than other organs. The order of organ examination depends on the priorities of your study. We usually examine liver and kidneys first. In small birds, the gall bladder may be studied without separation. In larger birds, however, it is best to separate the gall bladder from the liver and cut it open for examination in a separate small petri dish. Liver and kidneys need to be torn into small pieces, which can be done using scissors or tweezers (especially in the case of very small birds). However, we prefer to gently break apart liver and kidneys with gloved fingers, which preserves the ducts for examination and careful dissection of parasites, and reduces the probability of parasites being cut or damaged. Some dicrocoeliid digeneans from the liver (e.g., *Brachylecithum*, *Lutztrema*, etc.) and members of the family Eucotylidae from the kidneys can be tightly packed in the ducts and may not be easy to recover. The disrupted liver and kidneys of small birds can be examined immediately under a stereo-microscope. In case of larger birds (e.g., aquatic species), process the liver and kidney tissues using the same sedimentation method as described for the body wash – pour the disrupted liver or kidney tissues into a jar or a bottle with a lid, this time shaking the bottle, then pour the liquid into a beaker, allow for sedimentation to clear the supernatant, and retain the solids at the bottom. Repeatedly add new clean saline and carefully pour off until the supernatant is clear. Once clear, the solid contents at the bottom of the beaker can be examined. There is no need to shake tissues more than once. Examine the sediment in small portions under stereo microscope. Besides being the target organs for readily visible digeneans (e.g., Dicrocoeliidae and Opisthorchiidae in liver, Eucotylidae and Renicolidae in kidneys) the liver and kidneys are also important target organs to search for blood flukes. These can be extremely small, transparent, and are frequently fragmented, thus requiring particular attention during sediment screening.

The esophagus can be opened with scissors longitudinally. Some helminths, such as larger nematodes, can be readily seen and removed without optics, but the lumen and walls of the esophagus need to be examined under stereo-microscope. After the examination, you can compress the esophagus wall between two pieces of glass (size and thickness vary depending on the size of the bird), since some nematodes can be located in the wall and can be seen only under compression.

The stomach may contain representatives of several nematode families, digeneans, and even cestodes. The proventriculus and gizzard can be separated before examination. Stomach contents need to be removed and examined for the presence of helminths, nematodes in particular. Upon a preliminary examination, the proventriculus wall can be scraped with the edge of a microscope slide and the scraped material can be examined under the stereo-microscope. Nematodes, digeneans, and cestodes may be found under the lining of gizzard. In small birds, the gizzard wall lining can be easily peeled using forceps. In larger birds, especially large aquatic birds, peeling the gizzard wall can be more difficult, usually resulting in multiple fragments, and thus should be done in saline. After all of the lining is removed and rinsed, the liquid should be examined for helminths. The tapeworm genus *Gastrotaenia*, which are uniquely parasitic under the gizzard wall lining in anseriform birds, are small and easily mistaken for nematodes.

For the following steps, using scissors with at least one rounded/blunt/balled end (Figure 2.8 A–B) is strongly recommended. The duodenum and small intestine (Figure 2.8 C–D) typically contain the highest diversity and numbers of helminths. If blood flukes are among the targeted taxa, then examine the mesenteric veins and veins of the intestinal wall and cloaca for these parasites before opening the intestine or the cloaca.

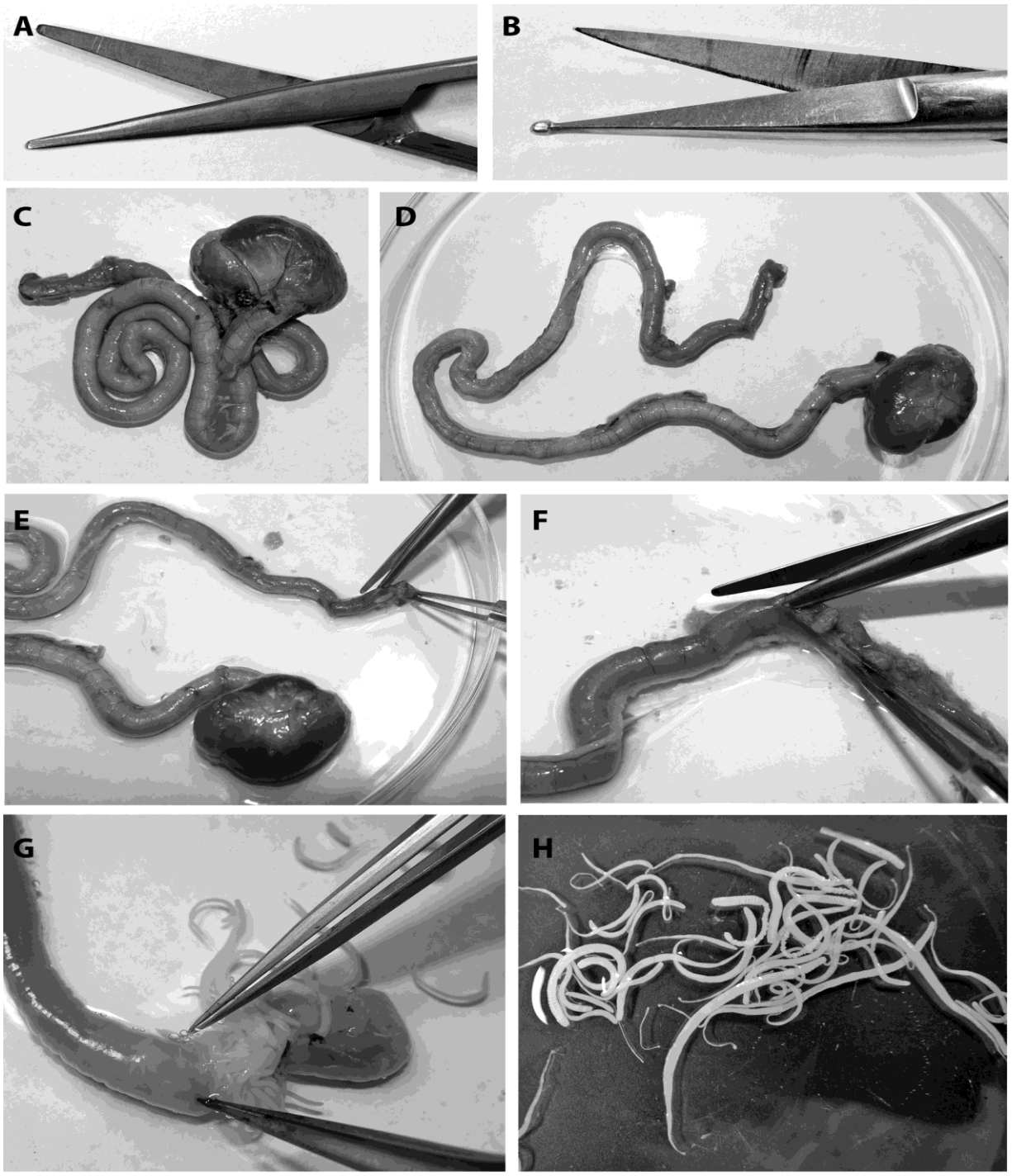

Figure 2.8. Dissection of the intestine of a small bird (American Robin *Turdus migratorius*). (A–B) Recommended scissors with rounded or balled ends (so-called "artery scissors"). (C) GIT removed from bird (esophagus was separated). (D) Straightened intestine with mesentery cut and fat removed. (E–F) Opening of the intestine with scissors starting from posterior end. (G) Gentle tearing of intestine using fine forceps after tapeworms were discovered. (H) Cestodes removed from intestine in saline.

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Next, one can remove excessive tissue (e.g., fat and connective tissue) around the intestine and open it with a longitudinal incision using scissors while holding the end of intestine with tweezers/forceps (Figure 2.8 E). For easier detection of tapeworm strobilae, it is best to begin the incision at the posterior end (Figure 2.8 F). If a tapeworm is detected, the subsequent dissection can be given more attention to find the scolex (or scoleces in case of numerous cestodes) buried in the mucous layer of the intestinal wall. After the entire intestine is open, it needs to be inspected for any other helminths readily visible to the naked eye or under a stereo-microscope (Figure 2.8 G). Acanthocephalans may be deeply embedded in the intestinal wall and need to be carefully removed. Any other visible helminths should also be removed and placed in a petri dish with saline (Figure 2.8 H). In some cases, this is not feasible due to very high number of helminths, especially if they are very small. To dislodge embedded helminths, scrape the intestinal wall with the side of a microscope slide under a shallow layer of saline solution. It is best to secure the end of the intestine with forceps, and it is essential to press the slide with sufficient force to scrape the intestinal wall deep enough to not break helminths that are deeply embedded in the layer of mucus. If the glass only slides on the surface of the mucus it is likely that some worms will be broken while others will remain attached to the intestinal wall in the mucus. Following this step, pour the saline with scrapings into a cylinder or bottle with a screw-cap, shake vigorously, and empty into a tall beaker (a water bottle with the top cut can be used in a field) and allow to settle for about two minutes (this time period depends on the density of the mix) before pouring off the supernatant. Sedimentation should follow the same basic procedure previously outlined. When the supernatant is clear, the sediment should be examined under the stereo-microscope or can be fixed with ethanol for subsequent examination in the lab (for a similar pocedure, see Justine et al. 2012).

Fixation of helminths

The choice of fixative for helminth fixation depends on the purpose and future use of the material. In cases when numerous individuals of the same species of helminths are available and time permits, it is always a good idea to use several fixatives, each optimized for a different downstream purpose. For example, morphological light microscopy, TEM, SEM, histology, immunology and molecular methods each require a different fixative. Some of these downstream procedures (e.g., TEM and immunological/immunohistochemical studies) require specialized fixatives, whereas others can use a common fixative (morphology, SEM and basic molecular analysis not involving NGS methods). Other than these, 70% ethanol is a fixative of choice, but with some limitations. Some authors prefer fixation of specimens for future staining with hot (steaming but not boiling) 10% formalin (i.e., 4% solution of formaldehyde) with subsequent storage in formalin. Others fix specimens in formalin with subsequent transfer to 70% ethanol for long-term storage. We have not noticed a significant difference in side-by-side comparisons of specimens simultaneously fixed with either fixative (and stained with the same stain). However, the use of heated formalin may have a negative impact on the researchers' health due to inhalation of toxic vapors. Furthermore, formalin hinders DNA extraction and subsequent molecular analyses. This is particularly important when considering that morphologically similar ("cryptic") species of helminths are not readily distinguishable in the field and may be present in samples. We therefore advocate the use of 70% ethanol as the single universal fixative equally suitable for "routine" morphological and molecular studies. It is also less hazardous and has fewer transportation restrictions than formalin.

We would like to emphasize that specimens fixed in ethanol (70% or 95%) need to be placed in a freezer or at least a refrigerator as soon as possible and stored long term in a freezer. In the lab of V. T. Tkach (co-author of this review), 80% ethanol is generally used as the starting concentration in the field, because during pipette specimen transfer to the storage vials, some fluid (water or saline) is inevitably added to the ethanol, which dilutes it to ~70%. Thus, by using 80% ethanol, we ensure that the concentration does not decrease significantly (which would result in poorly preserved specimens). It is recommended that you change ethanol once after the initial fixation to ensure sufficient concentration, though this is not always feasible during field collecting trips due to time and material limitations. As a general rule, for Next Generation Sequencing (NGS) and transcriptomic applications, freezing in liquid nitrogen is the gold standard of field preservation, followed by 95% ethanol for NGS and RNAlater (Sigma-Aldrich) for studies targeting RNA. Each group of helminths needs to be fixed using a slightly different protocol to properly preserve the morphological features of interest. The following are our recommendations for fixation of live worms using 70% ethanol.

Flatworms (digeneans and small to medium size cestodes) can be heat-killed with hot water. Remove most of the saline from the petri dish, leaving only very small amount of it to cover worms, in order to prevent even momentary desiccation. Pour hot water (steaming, not boiling) onto worms, stir the water using propulsion by pipette. Add ambient temperature water immediately to prevent overheating and transfer worms into vials with 70% ethanol (again, for practical reasons we use 80%) as soon as possible. In field conditions, a good quality thermos can be used to keep the water hot for some period of time rather than re-heating it every time one needs to heat-kill specimens. Change hot water as needed. Alternatively, flatworms can be pipetted into a petri dish or a small beaker with hot saline (see Cribb and Bray 2010) with

subsequent transfer to ethanol. In the case of very thick-bodied digeneans, a sub-sample can be fixed in ethanol under a slight cover slip or slide pressure (depending on the worm size). Such specimens can provide a better view of the organization of internal organs. However, specimens fixed in this manner may be distorted and should not be used to make measurements. Tapeworms should never be fixed under pressure. Very large tapeworms usually contract and become less suitable or completely unusable for morphological analysis if the above described heat-killing method is used. Thus, very large tapeworms may be killed and relaxed at the same time by moving them between a petri dish with water (ambient temperature) and a dish with ethanol of weak concentration (10%–15%) using a curved needle or curved tweezers to hold the tapeworm from underneath. Scoleces of large cestodes with armed rostellum can be fixed separately before the strobila is relaxed, to avoid loss of rostellar hooks. When the tapeworms die, they can be fixed in ethanol. We usually still "pre"-fix tapeworms in a petri dish before transferring them to a vial of appropriate size. The volume of ethanol in the vial should be at least 4–5 times greater than the volume of the tissue. Thus, with larger tapeworms, falcon tubes may be the preferred storage container, rather than vials.

Larger nematodes with a thick cuticle can be heat-killed following the general procedure outlined above. However, hot saline has to be used rather than water to prevent nematode bodies from rupturing due to the difference of osmotic pressure. Instead of hot saline being poured onto nematodes, the petri dish containing nematodes that have already been put in saline can be heated using an alcohol burner or lighter until the nematodes die. Heat the saline only to the point that it begins steaming and no further. The nematode cuticle may shrink somewhat in ethanol, even if it is only 70% ethanol. Higher concentration ethanol may distort nematodes irreversibly and is certainly not recommended for specimens to be used for future morphological

examination. Neutral buffered 10% formalin can be used for fixation and does not negatively affect morphology, but we tend to not use it for the reasons mentioned above. Small nematodes with a thin cuticle can be fixed with hot saline or hot 70% ethanol. In the latter case, they are simultaneously killed and relaxed. One must take care to prevent ethanol from catching on fire during heating. For an adequate morphological study of acanthocephalans, their proboscis should be fully everted. This is rarely achieved by heat-killing. Leaving them to die in water until their probosces are everted usually produces the best results. When possible, a dish containing water and acanthocephalans should be kept at a low temperature (e.g., in refrigerator), but even at ambient temperature (e.g., in a field camp) the desired result is usually achieved. Then, acanthocephalans can be transferred into ethanol.

All vials should have internal labels. Writing information with a sharpie on the outside of a label is not sufficient and is likely to result in the loss of data, which may render the specimens useless. Even vapors of ethanol inside a vial storage box may dissolve ink on the outside of the vials. Therefore, labels to be placed on the inside of the specimen vials need to be made of a paper that resists prolonged soaking in fixative without deterioration (archival acid free cotton fiber paper is best, but there are other alternatives), and should be written by hand or printed using either pencil or alcohol-proof ink or alcohol-resistant printer ink. In the latter cases, the ink needs to be tested prior to use, otherwise there is a risk of losing label information. Ethanol should fill almost all of the remaining space inside the vial leaving just a very small space for potential expansion at higher temperature. Leaving too much air in vials may result in dried specimens during the transportation of the vial boxes.

Preparing endoparasites for morphological study

The morphoanatomy of flatworms (digeneans and cestodes) is usually studied on permanent total microslide mounts. Parasitic nematodes are usually studied on temporary mounts and acanthocephalans can be studied on either permanent total mounts (mostly to study internal organs) or temporary mounts (mostly to study the proboscis armature and egg structure). There are a plethora of recipes for stains that have been used for trematode and cestode total mounts over the last hundred years. They can be found in numerous manuals and special publications (e.g., Dubinina 1971; Georgiev et al. 1986; Ivashkin et al. 1971; Pritchard and Kruse 1982). We refrain here from a discussion of advantages or disadvantages of one or another staining method. Instead, we provide only two stains and corresponding protocols that have been successfully used in Tkach's laboratory for a great variety of parasitic flatworms (Appendix B, C). Iron acetocarmine, Gill's hematoxylin, and Delafield's hematoxylin are useful alternatives that can be found in references provided above. It is important to realize that there is no one-size-fits-all staining procedure for every kind of specimen, and the amount of time and stain concentration may reasonably vary from taxon to taxon of flatworms. Staining and mounting is as much an art as it is science. Tkach's lab usually uses staining protocols with alum carmine (after Dubinina 1971, with minor modifications) and Mayer's haematoxylin (somewhat modified protocol used in Thomas Cribb's laboratory, e.g. Miller et al. 2010). Both stains are aqueous (water-based) and require specimens to be rinsed in water prior to staining.

Overview of parasite sampling workflow

In this section, we provide a condensed summary of the field workflow that is described in detail above. Based on our cumulative experience conducting comprehensive sampling of avian symbionts, we have listed steps in the order that we believe they are most efficiently and effectively performed. Of course, steps may be excluded (or introduced), depending on the research objectives and resources available.

Note: Be sure to record all data in the parasite field catalog, in addition to the standard voucher catalog.

Following capture of a live bird, carry out steps 1 – 5.

1. Assign the bird a field number, tissue number, or other unique identifier that will be recorded in the field notes of both the host and the parasites, and on the field sheets and field labels of the host and all of its parasites. Record soft-part colors on the field sheet (or directly into the host catalog).
2. Prepare small gauge needle and heparinized capillary tubes for quick manual access.
3. Hold bird in hand, with head and neck stabilized between index and third fingers (bander's grip), and wing extended and held between index and third (or third and fourth) fingers.
4. Place the tip of the needle parallel to the brachial vein, beveled side facing up.
5. Insert and withdraw quickly, followed by the collection of the beaded blood into the capillary tube(s).

Move on to step 10, while partner carries out steps 6 – 9.

6. Euthanize bird and attach leg tag with unique identifier.
7. Swab bird for microbiome analysis. Anatomical site of swabs will vary by research interest, but typically two swabs – buccal and cloacal – can be taken independently and placed in separate cryogenic tubes to be stored in liquid nitrogen (flash-frozen).

8. After swabbing, immediately place bird in a clean ziplock bag with cotton ball soaked in ethyl acetate (ectoparasite fumigant).
9. Apply small drop of blood from capillary tube to glass slide(s) and prepare blood smear(s).
10. Dab blood from capillary tube onto FTA card, and/or place directly into lysis buffer, absolute ethanol, or cryogenic tube (for flash-freezing storage in liquid nitrogen). Label all samples with the avian host's unique identifier (see Step 6).
11. After host has been fumigated for 10 – 15 minutes, remove from ziploc bag and ruffle for ectoparasites. Collect ectoparasites into vial containing 95% ethanol and label with host's unique identifier.
12. Record standard bird data (weight, molt, etc.) and enter into host voucher catalog.
13. Prepare host voucher specimen.
14. Once the skin isis removed from the host body, proceed to endoparasite sampling while host voucher preparation is completed by partner.

Conclusion

For ornithologists who do not intend to study parasites or pathogens directly, or who do not have the facilities to curate their specimens, there are many options for ensuring that specimens find their way into collections where they will be curated and utilized. We strongly recommend that type and voucher specimens be deposited in museum collections. In the United States, the main collections curating helminth and other endoparasite specimens and providing loans for studies are the U.S. National Parasite Collection (now a part of the Smithsonian Institution), Harold W. Manter Laboratory of parasitology at the University of Nebraska,

Lincoln, and the parasite collection of the Museum of Southwestern Biology, Albuquerque, New Mexico. Most museums with large entomological holdings will also house collections of arthropod ectoparasites and are appropriate places for depositing this material. There are, of course, a large number of other parasitological collections both in the U.S. and around the world. We recommend submission of specimens to museums that provide specimen loans for examination. Several publications (e.g., Lamothe-Argumedo et al. 2010; Lichtenfels and Pritchard 1982; Zinovieva et al. 2015) provide useful information on the location and scope of taxonomic coverage of most important helminth museum collections.

ACKNOWLEDGEMENTS

We would like to thank the organizers of the 143rd American Ornithological Union conference and the organizers of the symposium in which our comprehensive sampling workflow was first publicly presented. Specifically, we thank Mike Webster and Kim Bostwick for their commitment to spreading the idea of "the extended specimen". We would also like to thank our many colleagues who have helped support, implement, and refine many of the protocols in our workflow, including Josh Engel, Tom Gnoske, John Bates, Shannon Hackett, and Nate Rice. For photographs of ectoparasites, we thank Jason Weintraub (Academy of Natural Sciences of Drexel University), Michael W. Hastriter (Monte L. Bean Life Science Museum, Brigham Young University), Michel P. Valim (Museu do Zoologia da Universidade de São Paulo), Lorenza Beati (U.S. National Tick Collection, Georgia Southern University), and Fabio Akashi Hernandes (Universidade Estadual de São Paulo). This work was supported in part by the U. S. National Science Foundation (grants DEB-1503804 (1120054) to JDW, and DEB-1120734 and DEB-1021431 to VVT). This work has also been supported by grants to HLL from the NSF Malaria Research Coordination Network and the Cornell Lab of Ornithology's Athena Fund.

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CHAPTER 3

PARASITE PREVALENCE CORRESPONDS TO HOST LIFE HISTORY IN A DIVERSE ASSEMBLAGE OF AFROTROPCAL BIRDS AND HAEMOSPORIDIAN PARASITES[2]

Abstract

Avian host life history traits have been hypothesized to predict rates of infection by haemosporidian parasites. Using molecular techniques, we tested this hypothesis for parasites from three haemosporidian genera (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) collected from a diverse sampling of birds in northern Malawi. We found that host life history traits were significantly associated with parasitism rates by all three parasite genera. Nest type and nest location predicted infection probability for all three parasite genera, whereas flocking behavior was an important predictor of *Plasmodium* and *Haemoproteus* infection and habitat was an important predictor of *Leucocytozoon* infection. Parasite prevalence was 79.1% across all individuals sampled, higher than that reported for comparable studies from any other region of the world. Parasite lineage diversity was also exceptionally high, with 248 parasite lineages identified from 152 host species. A large proportion of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasite DNA sequences identified in this study represent new, previously undocumented lineages (n = 201; 81% of total identified) based on BLAST queries against the avian malaria database, MalAvi.

[2] Lutz HL, Hochachka WM, Engel JI, Bell JA, Tkach VV, Bates JM, Hackett SJ,Weckstein JD. 2015. Parasite prevalence corresponds to host life history in a diverse assemblage of Afrotropical birds and haemosporidian parasites. Plos One. 10(4): e0121254.

Introduction

Vector-borne pathogens are responsible for a vast number of diseases that negatively impact animal and human health. Many of these pathogens can infect multiple hosts, and prevalence varies among susceptible host species (Ewald 1983; Poulin 2007). Although factors contributing to variation in host susceptibility are complex (Combes 1997; Dobson 2004; Lafferty 2009; Medeiros et al. 2013), host-vector encounter rates are known to play an important role in the transmission of some vector-borne pathogens (Janousek et al. 2014; Kilpatrick et al. 2006). Host life history traits and behaviors have been associated with host–vector encounter rates (Blackmore and Dow 1958; Edman and Kale 1971), and may be an important filter for transmission of vector-borne pathogens such as malarial parasites. Empirically identifying which life history traits have the greatest filtering effects on transmission within vector-borne disease systems will improve epidemiological models (Dobson 2004), as well as our understanding of the fundamental ecological and evolutionary mechanisms underlying pathogen virulence, prevalence within populations, and host susceptibility across taxa.

Avian models have provided important insights into the relationships between host life history traits and transmission of vector-borne pathogens such as the bacterial Lyme disease agent *Borrelia burgdorferi* (Giardina et al. 2000; Ogden et al. 2008), St. Louis encephalitis (Mahmood et al. 2004), West Nile virus (WNV) (Gilbert 2012; Janousek et al. 2014; Kilpatrick et al. 2006), Western equine encephalomyelitis (Hayes et al. 1967), and Eastern equine encephalitis (Unnasch et al. 2006). Among other host life history traits, those related to nesting behavior may be particularly relevant to parasite transmission. For altricial nestlings, a

combination of naïve immune system, bare skin exposed by poor feather coverage, and a stationary position in the nest are generally expected to increase susceptibility to vector-borne pathogens (Blackmore et al. 1958; Edman and Kale 1971; Edman and Scott 1987; Kale et al. 1972; Scott and Edman 1991). Likewise, adult hosts, due to their relatively stationary position during brooding and the accumulation of chemical cues for the vectors of pathogens, may be more highly exposed (Burkett-Cadena et al. 2010; Scott et al. 1990). For example, a "host funnel" for WNV at the end of the nesting season produces an increase in contact rates between the ornithophilic vectors, *Culex* spp. mosquitoes (Diptera: Culicidae) and nesting birds (Caillouët et al. 2013), resulting in amplification of the pathogen (Day and Stark 1999).

Avian haemosporidian parasites (phylum Apicomplexa) have served as important models for the study of host–parasite interactions (Bennett 1960; Bennett and Fallis 1960; Garvin and Remsen 1997; Ricklefs 1992), parasite-mediated selection (Hamilton and Zuk 1982; Pruett-Jones et al. 1991; Read 1988; Scheuerlein and Rickleffs 2004), and genetics and epidemiology of human malaria (Atkinson and van Riper III 1991; Jasinskiene et al. 2007; Miller et al. 2002). Avian haemosporidia, which include malaria parasites (*Plasmodium*) and closely-related genera (*Haemoproteus*, *Leucocytozoon*), maintain labile associations with vertebrate hosts through time and space (Beadell et al. 2009; Fallon et al. 2004; Ishtiaq et al. 2007). Diversity among haemosporidian parasites has been drastically underestimated by microscopy-based studies (Bensch et al. 2004; Valkiūnas 2005) and a growing number of molecular studies have now identified over 1,500 unique parasite lineages (Beadell et al. 2009; Belo et al. 2011; Chasar et al. 2009; Fecchio et al. 2013; Ishtiaq et al. 2012; Lacorte et al. 2013; Loiseau et al. 2010; Loiseau et al. 2011; Svensson-Ceolho et al. 2013).

Tropical bird communities and their haemosporidian parasites are a good model system for investigating the role of avian life history traits on probabilities of infection with vector-borne pathogens. Tropical bird species occupy a broad range of habitats, often in close proximity to each other, and exhibit a wide variety of flocking behaviors, habitat preferences, nest types, and nest placements. This system is therefore suitable for testing the ability of certain host life history traits to predict rates of parasitism across a broad spectrum of hosts. Furthermore, improved sampling of little-studied tropical avian haemosporidia, particularly outside of the Western Hemisphere, which have been poorly studied relative to their temperate counterparts, has important implications for our ability to understand haemosporidian ecology and evolution on a broader biogeographic scale (Clark et al. 2014).

In this study, we tested the hypothesis that life history traits of Afrotropical birds predict rates of parasitism by three haemosporidian parasite genera (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*). We included, in our analyses, traits that are known to be associated with host–vector encounter rates – nest type, nest placement, and flocking behavior. We have combined broad taxonomic sampling of host species from a wide variety of habitats and life histories in Northern Malawi with the application of rigorous PCR-based methods to detect rates of parasitism. Additionally, we describe an unprecedented number of novel parasite lineages and their associations with birds in Malawi.

Methods

Field Sites and Sampling

Sampling was carried out from October to November, 2009, in Vwaza Marsh Wildlife Reserve and Nyika National Park in Malawi (Figure 3.1) as part of a larger project to assess parasite and pathogen diversity in African birds. Birds were sampled from a variety of habitats at each field site (Engel et al. 2012) to thoroughly assess host and parasite diversity (Table 3.1). For statistical analyses, these habitats were grouped into five broad classes: aquatic, evergreen forest, forest edge, grassland/marsh, and riparian forest/woodland. Host voucher specimens were collected and deposited at the Field Museum of Natural History (Chicago, IL) and the Museums of Malawi (Blantyre, Malawi). The Malawi Department of Forestry (License No. 3/12/2007/1, granted on 10 July, 2009, valid from 10 July, 2009 to 10 July, 2010) and the Department of National Parks and Wildlife (Ref. No. NPW/2/1/12, granted on 6 October, 2009, valid from October to November, 2009) provided permits for collecting vertebrate specimens in Vwaza Marsh Wildlife Reserve (Base camp: 11˚08.03'S, 33˚39.31'E; elevation 1092 m) and Nyika National Park (Base camp: 10˚73.33'S, 33˚96.67'E; elevation 2233 m). Birds were euthanized via thoracic compression following the euthanization guidelines published in the American Ornithologists' Union's "Guidelines to the Use of Wild Birds in Research" (http://www.nmnh.si.edu/BIRDNET/guide/). The protocols described in this document were approved for use by the Field Museum's Institutional Animal Care and Use Committee. We did not collect specimens of endangered or threatened species, and we note that avian specimens are being used in ongoing morphological studies of geographic variation in African birds.

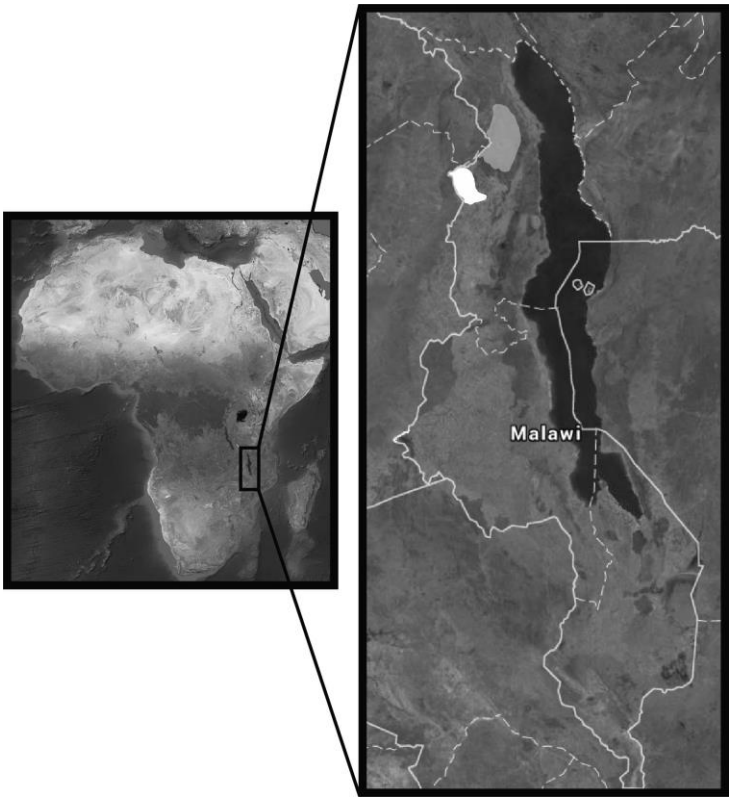

Figure 3.1. Map of sampling locations in northern Malawi. Vwaza Marsh Wildlife Reserve, white; Nyika National Park, gray. Image credit: USGS National Map Viewer.

Table 3.1. Sampling localities and habitat types in Malawi

Field Site	Habitats Sampled
Vwaza Marsh Wildlife Reserve	Lake edge
Latitude: 11˚ 08.033’ S	Marsh
Longitude: 33˚ 39.307’ E	Miombo woodland
Elevation: 1071 – 1170 m	Riparian woodland
Nyika National Park	Evergreen forest
Latitude: 10˚ 35.307’ S	Miombo woodland
Longitude: 33˚ 48.670’ E	Montane grassland (open)
Elevation: 1647 – 2347 m	Montane grassland (scrub-lined watercourse)
	Riparian woodland

In total, we sampled 532 birds from 16 orders, 50 families, 100 genera, and 152 species. Blood was collected via brachial venipuncture from live birds and stored on Whatman FTA Classic Cards (GE Healthcare, Piscataway, NJ). Of the 532 birds originally sampled, data from 469 individuals were included in our statistical analyses of host life history traits, after removing data from the following: Palearctic migrants that do not nest in Africa, nest-parasitic species for which we could not classify nest height, and domestic species (i.e., *Gallus gallus domesticus*). We also excluded individuals sustaining coinfections from our analyses when parasite identities could not be differentiated. Lastly, "aquatic" host species were removed from analyses of *Haemoproteus* and *Leucocytozoon* parasitism rates, as none of these host individuals were infected by these two parasite genera. This was necessary because the models containing habitat as a predictor did not converge to solutions when no variation in response was found within this habitat class.

Molecular detection of haemosporidian parasites

Parasites were detected via replicated applications of a nested polymerase chain reaction (PCR) method based on Hellgren et al. (2004) and Waldenström et al. (2004). Genomic DNA was extracted from whole blood stored on Whatman FTA Classic Cards using Qiagen Blood and Tissue Mini kits, following the protocol for dried blood spots (Qiagen, Valencia, CA). Parasitemia, or the quantity of parasites circulating in an individual host, can vary highly between patent and chronic stages of infection. Haemosporidian infections in birds can persist in a chronic stage for years (Fallon et al. 2003), and therefore the starting quantity of parasite DNA

in blood samples may be low, leading to a high occurrence of false negatives (Freed and Cann 2006; Richard et al. 2002). We applied PCR protocols multiple times to all individual samples, which had the effect of increasing probability of detecting low-level infections that were not found in all of the multiple repeated tests. To our knowledge, our application of triplicate screens is a novel approach for improving detection of haemosporidian parasites from blood samples. For detection of *Plasmodium* and *Haemoproteus,* PCR screens targeting the mitochondrial cytochrome *b* gene (*cytb*) were conducted three times from the extracted DNA using identical conditions, and for detection of *Leucocytozoon*, PCR screens targeting *cytb* were conducted using each of two sets of *Leucocytozoon*-specific primers a single time (Appendix D). Negative controls were included in all PCR runs to control for false positives due to the high sensitivity of nested PCR (*sensu* Hellgren et al. 2007). PCR products identified as positive for haemosporidian infection were prepared for direct sequencing by enzymatic ExoSAP-IT (Affymetrix) or gelase b-agarose gel-digesting purification (Epicentre Technologies, Madison, Wisconsin). Purified PCR products were sequenced using ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase FS (Perkin-Elmer) and run on an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, California) using both the forward and reverse primers HAEMF and HAEMR2 for *Plasmodium* and *Haemoproteus*, and either HAEMFL and HAEMR2L (Hellgren et al., 2004) or L545F and L825R (this study; Appendix D) for *Leucocytozoon*. Sequence data were edited using Sequencher v.5.0.1 (GeneCodes, Ann Arbor, Michigan) and Geneious v.6.1.6 (Biomatters).

Although microscopic methods may be less sensitive than molecular methods (Richard et al. 2002; Valkiūnas et al. 2008), we also visually screened blood films for the presence of malarial parasites. Blood films for 431 of the 532 birds sampled were prepared in the field and

fixed with 100% methanol. Blood films were then stained with Giemsa and screened for infection by *Plasmodium, Haemoproteus,* or *Leucocytozoon* (*Haemoproteus* and *Plasmodium* identifications were scored without distinguishing between genera). Blood films were screened under 1000× magnification, and 100 fields were screened for each film. Blood films from individuals that screened positive for haemosporidian infection based on PCR, but were negative by the initial round of microscopy, were subjected to an additional round of microscopic screening at 1000×. In the second round of microscopy, 200 fields were examined for each film. We found molecular methods to be more sensitive, with false negatives occurring in < 0.2% of molecular screens with respect to microscopically identified positives, whereas false negatives occurred in > 37% and > 55% of microscopic screens for *Leucocytozoon* and *Plasmodium*/*Haemoproteus* infections respectively. Thus, we chose to rely solely on infections detected by molecular methods for our statistical analyses.

Identification of haemosporidian parasite lineages

In cases where multiple parasite lineages were detected within one individual host, unique DNA sequences were considered to correspond to individual parasites (i.e., if an individual screened positive for the presence of malaria parasites two out of three times, both PCR products were sequenced; if these sequences differed, and did not contain ambiguities or double peaks, they were considered unique parasite lineages that co-infected the same host). If data from any positive PCR contained sequence ambiguities that could not be differentiated (i.e., two or more clean double peaks on the chromatogram), the host was scored as having an ambiguous coinfection. Relatively few parasite sequences were ambiguous (< 12% of total

coinfections detected) after performing multiple rounds of PCR amplification and sequencing. Ambiguous sequences and associated host data were removed from subsequent statistical analyses.

Plasmodium, *Haemoproteus,* and *Leucocytozoon* sequences were collapsed to unique haplotypes, which were then subjected to a BLAST search against the MalAvi database (Bensch et al., 2009) to identify parasite lineages. Lineages previously identified in the MalAvi database (100% pairwise identity compared to known sequences) were named accordingly, and novel lineages from this study (< 100% pairwise identity compared to published sequences) were assigned names prefixed according to geographic location (Africa = "AFR" in Appendix E). Lineages identified in this study are available both on GenBank (Accession Numbers KM056404 – 056650) and the MalAvi database (Bensch et al. 2009).

Selection and scoring of life history and ecological parameters for host species

We included life history traits in our models that are linked to host–vector encounter rates during the nesting period (e.g., nest type, and nest height) because the blood parasites in our study are vector-borne and nestlings have been shown to be more vulnerable to host-seeking vectors (Blackmore et al. 1958; Edman and Kale 1971; Edman and Scott 1987; Kale et al. 1972; Scott and Edman, 1991). We also included host flocking behavior, because of the known role of kairomones (olfactory cues emitted by hosts) in attracting the dipteran hosts of haemosporidian parasites (Logan et al. 2010; Wickler et al. 1980; Withers 1978), and habitat, to account for variation of haemosporidian and vector prevalences at sampled sites. Nest type was categorized as open cup, closed cup, or cavity. Closed cup includes nests other than cavities that are built

with a covering, creating an enclosed chamber, such as pendant or spherical nests built by weavers. Nest location was categorized as ground, understory, canopy/sub-canopy, or cliff/bank. Species were classified as having understory nests if nests occur predominantly < 3 meters above the ground; nests above 3 meters were classified as canopy/sub-canopy. Flocking behavior was categorized as solitary (species that predominantly forage singly or in pairs, e.g., Cuculidae); single-species flock (including species that forage primarily as family groups, e.g., Plocepasser rufocapulatus, or species that form larger single-species flocks, e.g., Lamprotornis chalybeus); or mixed-species flock (e.g., Parus and many other forest and woodland passerines). Habitat was categorized as described in methods. Parameter scores for all species sampled in our study can be found in Appendix F. Data for these parameters were obtained from The Birds of Africa series (Brown et al. 1982; Fry et al. 1988; Urban et al. 1986; Keith et al. 1992; Urban et al. 1997; Fry et al., 2002; Fry et al. 2004). Four species in our study lacked nesting data (Cisticola njombe, Serinus citrenilloides, Serinus striolatus, Laniarius fuelleborni). For these species, nesting habits are consistent for all members of their respective genera, so values were inferred from their close relatives. Species that commonly occur in multiple habitats (e.g., Zosterops senegalensis) were scored according to the habitat from which they were collected in our study.

Statistical Analyses

We used generalized linear mixed models to identify which combination of host life history, behavioral, and ecological factors best predicted the probability of an individual bird being parasitized. Independently for each parasite genus (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), we assessed the ability of 15 different logistic regression models (Table 3.2) to

predict the binomial response variable, uninfected vs. infected. All fixed effects — nest type, nest location, flocking behavior, and habitat — were treated as categorical variables. To account for host phylogenetic constraints on parasitism due to factors that we did not measure, we included three nested random effects: host family, host genus nested within host family, and host species nested within host genus nested within host family (Table 3.3). This approach allowed us both to account for statistical non-independence in our data owing to host phylogenetic constraint, and to identify the taxonomic level at which these unexplained effects (if any) were occurring. Because our samples came from two sites at different elevations during different months – Vwaza Marsh Wildlife Reserve (1071–1170 m; October) and Nyika National Park (1647–2347 m; November) – we statistically controlled for any differences in baseline levels of parasitism between sites/months by including site as a fixed effect in all of our analyses.

Table 3.2. Fixed effects in the set of 15 models and the relative support of data for these models (ΔAIC_C values) from AIC-based multi-model comparisons. We used these comparisons to identify life history traits associated with rates of haemosporidian parasitism of avian hosts. An "X" indicates that a given trait (column) was used as a fixed-effect categorical variable in a given model (row). For each parasite genus, the model with the ΔAIC_C value of zero is the best-supported model.

Model #	Nest Location[a]	Nest Type[b]	Flocking Behavior[c]	Habitat[d]	Study Site[e]	ΔAIC_C *Plasmodium*	ΔAIC_C *Haemoproteus*	ΔAIC_C *Leucocytozoon*
1	X				X	5.224	2.435	11.471
2		X			X	1.852	3.096	24.121
3			X		X	0.222	5.594	22.932
4				X	X	7.633	10.063	11.162
5	X	X			X	2.276	1.680	12.450
6	X		X		X	3.690	0.662	12.770
7	X			X	X	11.716	8.230	1.939
8		X	X		X	0	2.539	24.176
9		X		X	X	7.274	6.723	9.258
10			X	X	X	6.020	7.331	13.148
11	X	X	X		X	0.744	0	14.211
12	X	X		X	X	13.336	5.140	0
13	X		X	X	X	10.380	4.579	5.262
14		X	X	X	X	4.615	3.951	12.133
15	X	X	X	X	X	6.896	4.038	3.719

[a]Nest Location: Ground, Understory, Canopy/Sub-canopy, Cliff or bank

[b]Nest Type: Open cup, Closed cup, Cavity

[c]Flocking Behavior: Solitary, Monospecific flock or family group, Mixed-species flock

[d]Habitat: Woodland, Grassland or Marsh, Forest edge, Aquatic, Evergreen forest

[e]Breeding Social Behavior: Colonial, Solitary

[e]Study Site: Vwaza Marsh Wildlife Reserve, Nyika National Park

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Table 3.3. Tests of statistical significance of host phylogenetic constraints on probability of parasitism. Phylogenetic effects were examined in our analyses by including nested random effects of host family, genus (within family), and species (within genus) on the probabilities of parasitism with each of the three genera of parasites. Statistical tests were likelihood ratio tests each with a single degree of freedom.

Parasite genus	Host taxonomic level	Chi-squared value	P-value
Plasmodium			
	Family	4.53	0.03
	Genus (within Family)	0.00	1
	Species (within Genus)	0.00	1
Haemoproteus			
	Family	0.00	0.97
	Genus (within Family)	1.27	0.26
	Species (within Genus)	6.40	0.01
Leucocytozoon			
	Family	0.30	0.59
	Genus (within Family)	1.82	0.18
	Species (within Genus)	0.00	0.97

Our main conclusions are based on the approach to model comparisons and weighted averaging outlined by Burnham and Anderson (2002). Models were ranked by importance based on weights calculated using Akaike's Information Criterion (AIC) (Table 3.4). We assessed the relative importance of each fixed-effect predictor variable by calculating the cumulative support for each predictor as the sum of weights of all models containing that predictor. The effect of each predictor and its precision were estimated by calculating weighted average ("model-averaged") regression coefficients, standard errors, and 95% confidence limits (Table 3.5). To make qualitative comparisons among all categories, we produced graphs illustrating the size of each effect (see below) for which we found significant regression coefficients (i.e., coefficients with model-averaged confidence limits not overlapping zero).

Table 3.4. AIC-based support for fixed effects. Values are sums of model weight values for all models in the set (Table 3.2).

Fixed Effect	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*
Nest type	0.68	0.65	0.72
Nest location	0.34	0.78	0.99
Flocking Behavior	0.78	0.70	0.14
Habitat	0.06	0.15	1

Although our major conclusions are based on the multi-model procedures outlined above, two additional results are based on examining output from single models. First, to display variation in the expected probabilities of parasitism for each haemosporidian genus, we calculated least-squares mean probabilities of parasitism from the single model in each set that contained all of the predictor variables identified as important based on model-averaged coefficients and their confidence intervals (see previous paragraph). Second, we used these same models (one for each haemosporidian genus) to model parasitism rates, then calculated the statistical significance of each of the three random effects used to account for unexplained host phylogenetic constraints. The significance of each random effect was determined using a likelihood ratio test that compared the full model (all fixed and random effects present) with a model in which only the focal random effect was removed from the full list.

Table 3.5. Model-averaged regression coefficients, standard errors, and 95% confidence limits used to estimate effects of predictors and precision of effects. Note that for each predictor, the regression coefficients are interpreted as describing deviations in parasitism rates from a reference category whose effect is subsumed into the intercept term of the statistical model. Thus, although we can compare parasitism rates of understory nesting species with ground nesting species (the reference category for nest location) using the model-averaged regression coefficients, we cannot use these coefficients to directly compare parasitism rates of understory and canopy nesters.

		Plasmodium Model-averaged beta, 95% confidence limits			*Haemoproteus* Model-averaged beta, 95% confidence limits			*Leucocytozoon* Model-averaged beta, 95% confidence limits		
Parameter	Parameter description	Beta	Low. CL	Upp. CL	Beta	Low. CL	Upp. CL	Beta	Low. CL	Upp. CL
(Intercept)		-1.53	-2.49	-0.57	-1.14	-2.64	0.37	-3.48	-5.21	-1.74
Nest Location	Understory	0.38	-0.38	1.14	0.39	-0.81	1.60	1.83	0.64	3.02
Nest Location	Canopy/Subcanopy	0.62	-0.29	1.52	1.55	0.28	2.83	2.35	0.97	3.72
Nest Location	Cliff/Bank	1.94	0.07	3.81	0.51	-1.97	2.99	-0.49	-3.32	2.34
Nest Type	Closed cup	0.71	0.06	1.36	-1.12	-2.07	-0.17	-0.28	-1.18	0.62
Nest Type	Cavity	-0.24	-1.08	0.6	0.42	-0.65	1.50	1.38	0.19	2.57
Flocking Behavior	Same-species flock or family group	0.5	-0.12	1.12	-0.87	-1.75	0.02	-0.12	-0.94	0.69
Flocking Behavior	Mixed-species flock	0.89	0.17	1.61	-1.28	-2.32	-0.23	0.19	-0.74	1.12
Habitat	Grassland/Marsh	-0.27	-1.07	0.54	-0.37	-1.56	0.83	1.67	0.37	2.97
Habitat	Forest edge	-0.02	-0.77	0.73	0.27	-0.74	1.28	1.47	0.30	2.64
Habitat	Aquatic*	-0.68	-3.29	1.94	NA	NA	NA	NA	NA	NA
Habitat	Evergreen forest	-0.53	-1.44	0.38	0.52	-0.67	1.71	2.49	1.25	3.73
Site	Vwaza Marsh Wildlife Reserve	0.84	0.36	1.31	-0.08	-0.84	0.68	-1.38	-2.13	-0.62

*Aquatic habitat was only included as a fixed effect for *Plasmodium* analysis, as zero individuals from aquatic habitats were infected by *Haemoproteus* or *Leucocytozoon.*

All models were fit using restricted maximum likelihood implemented with the glmer function from the lme4 package (Bates et al. 2012) within R (version 3.0.2; R Development Core Team 2013). Model weights and model-averaged regression coefficients were calculated using the aictab.mer and modavg.mer functions found in the R package AICcmodavg (Mazerolle 2013). We used the R package lsmeans to calculate least-squares means and their confidence intervals. The statistical significance of host phylogeny (random effects) was calculated with Chi-squared likelihood ratio tests using the rand function in the lmerTest package (Kuznetsova et al. 2013) within R.

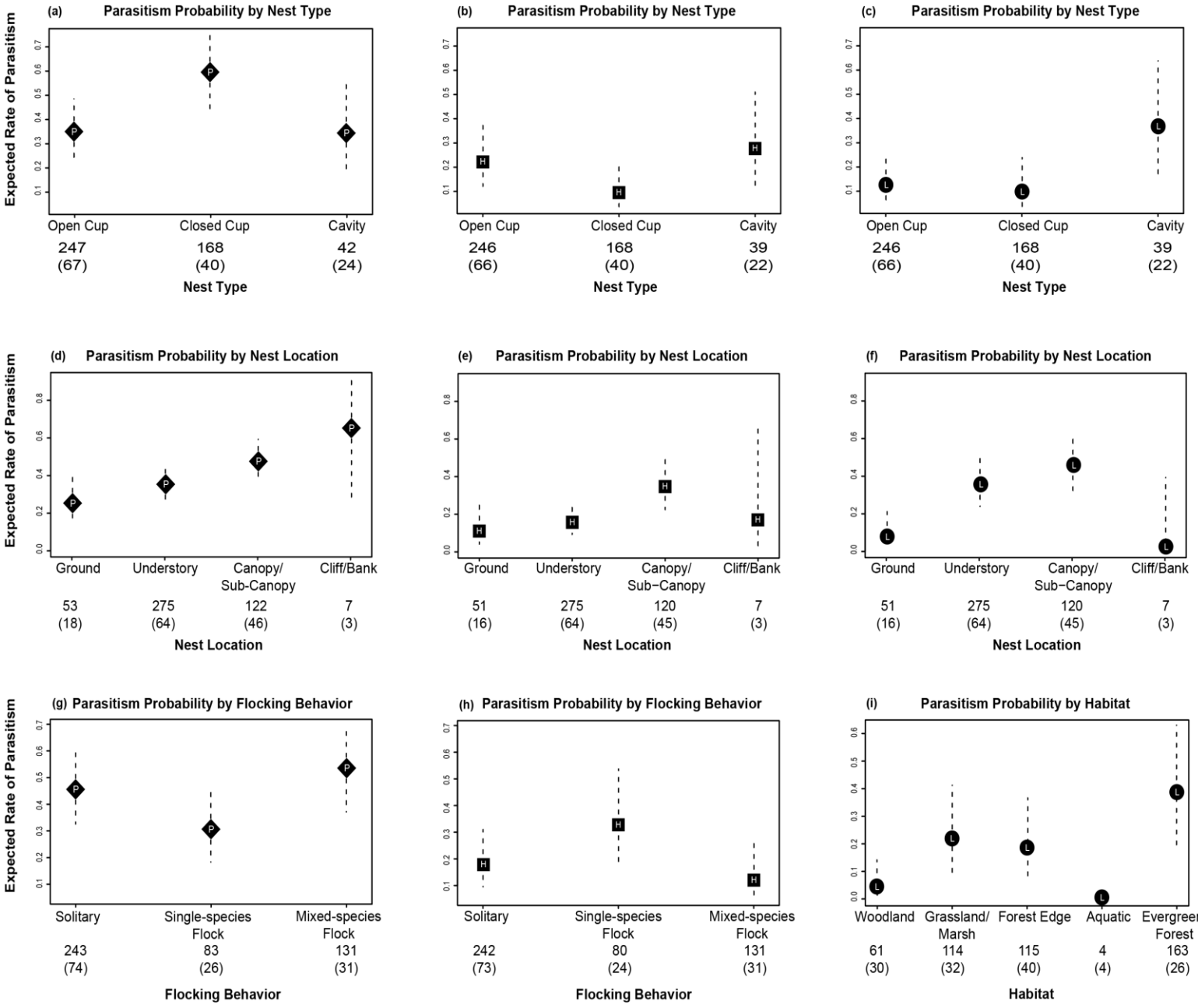
Results

General patterns, model selection and effect of life history traits on rates of parasitism

Host nest type and location were important predictors of infection for all three parasite genera (Table 3.5). We also found that host flocking behavior was an important predictor of *Plasmodium* and *Haemoproteus* infections, and that habitat was an important predictor of infection by *Leucocytozoon* only (Figure 3.2 a–i). In general, cisticolas (Cisticolidae), weavers (Ploceidae), and estrildid finches (Estrildidae), all of which build closed cup nests and thrive in a range of habitats, were frequently parasitized by *Plasmodium* (Table 3.6). Greenbuls (Pycnonotidae) and white-eyes (Zosteropidae), on the other hand, experienced low rates of parasitism by *Plasmodium* and high rates of parasitism by *Leucocytozoon* and *Haemoproteus*. Pigeons and doves (Columbidae) sampled in this study were primarily parasitized by *Haemoproteus* parasites in the subgenus *Haemoproteus* (unpublished molecular analyses). However, one individual (African olive pigeon) was parasitized by a novel *Parahaemoproteus*

lineage that was most closely related to the strigiform parasite *Haemoproteus syrnii* (subgenus *Parahaemoproteus*). Notably, no nightjars (Caprimulgiformes) sampled in this study (n = 8) were parasitized. The Caprimulgiform species sampled included the Fiery-necked nightjar (*Caprimulgus pectoralis*), the Square-tailed nightjar (*Scotornis fossii*), and the Ruwenzori nightjar (*Caprimulgus poliocephalus*), all of which are solitary, open cup, ground-nesting species. Host–parasite association data for all individuals sampled are summarized in Appendix E.

Based on confidence limits around the model-averaged regression coefficients, infection by *Plasmodium* was significantly higher in closed cup nesting birds (n = 168 individuals sampled; 40 host species) relative to cavity-nesting (n = 39 individuals sampled; 22 host species) or open cup nesting host species (n = 247 individuals sampled; 67 host species) (Figure 3.2 a–c). In contrast, infection by *Haemoproteus* was significantly lower in closed cup nesting birds relative to cavity-nesting or open cup nesting host species. Lastly, cavity-nesting species had a higher probability of *Leucocytozoon* infection than either the open cup or closed cup nesting host species.


(a) Parasitism Probability by Nest Type
Expected Rate of Parasitism
Open Cup
Closed Cup
Cavity
247 (67)
168 (40)
42 (24)
Nest Type
(b) Parasitism Probability by Nest Type
Open Cup
Closed Cup
Cavity
246 (66)
168 (40)
39 (22)
Nest Type
(c) Parasitism Probability by Nest Type
Open Cup
Closed Cup
Cavity
246 (66)
168 (40)
39 (22)
Nest Type
(d) Parasitism Probability by Nest Location
Expected Rate of Parasitism
Ground
Understory
Canopy/ Sub-Canopy
Cliff/Bank
53 (18)
275 (64)
122 (46)
7 (3)
Nest Location
(e) Parasitism Probability by Nest Location
Ground
Understory
Canopy/ Sub−Canopy
Cliff/Bank
51 (16)
275 (64)
120 (45)
7 (3)
Nest Location
(f) Parasitism Probability by Nest Location
Ground
Understory
Canopy/ Sub−Canopy
Cliff/Bank
51 (16)
275 (64)
120 (45)
7 (3)
Nest Location
(g) Parasitism Probability by Flocking Behavior
Expected Rate of Parasitism
Solitary
Single-species Flock
Mixed-species Flock
243 (74)
83 (26)
131 (31)
Flocking Behavior
(h) Parasitism Probability by Flocking Behavior
Solitary
Single-species Flock
Mixed-species Flock
242 (73)
80 (24)
131 (31)
Flocking Behavior
(i) Parasitism Probability by Habitat
Woodland
Grassland/ Marsh
Forest Edge
Aquatic
Evergreen Forest
61 (30)
114 (32)
115 (40)
4 (4)
163 (26)
Habitat

1640

1641

Figure 3.2. Predicted (least-squares mean) probabilities of parasitism and their 95% confidence intervals. Expected rates of parasitism illustrated according to (a – c) host nest type, (d – f) host nest location, (g – h) host flocking behavior, and (i) habitat. For all panels, *Plasmodium* is represented by a black diamond and the letter "P", *Haemoproteus* is represented by a black square and the letter "H", and *Leucocytozoon* is represented by a black circle and the letter "L". Number of individuals and species comprising each trait are listed below their respective traits (number of individuals above, number of species below in parentheses). Note that the parasitism rate of zero has been plotted for the aquatic habitat without confidence intervals because the four individual aquatic-habitat birds sampled lacked *Leucocytozoon* infections and therefore could not be used in the statistical analysis (as noted in the Methods section) Thus, no measure of statistical confidence is associated with this aquatic habitat plotted point

Table 3.6. Rates of parasitism by higher level avian taxa (Order and Family)

Host Order	Host Family	Host Species (n)	Host Samples (n)	*Plasmodium* Infected (n)	*Plasmodium* Infected %	*Haemoproteus* Infected (n)	*Haemoproteus* Infected %	*Leucocytozoon* Infected (n)	*Leucocytozoon* Infected %	Uninfected (n)	Uninfected %	Genus Unknown (n)	Genus Unknown %
Anseriformes	Anatidae	2	3	1	33%	0	0%	0	0%	2	67%	0	0%
Bucerotiformes	Bucerotidae	2	2	1	50%	0	0%	2	100%	0	0%	1	50%
Caprimulgiformes	Caprimulgidae	3	7	0	0%	0	0%	0	0%	7	100%	0	0%
Ciconiiformes	Ardeidae	2	3	0	0%	2	67%	0	0%	0	0%	1	33%
	Scopidae	1	1	0	0%	1	100%	1	100%	0	0%	0	0%
Coliiformes	Coliidae	1	2	1	50%	0	0%	0	0%	1	50%	0	0%
Columbiformes	Columbidae	5	10	0	0%	7	70%	1	10%	3	30%	0	0%
Coraciiformes	Alcedinidae	3	5	1	20%	3	60%	1	20%	1	20%	1	20%
	Coraciidae	1	1	0	0%	0	0%	1	100%	1	100%	0	0%
	Meropidae	2	8	1	13%	3	38%	1	13%	4	50%	0	0%
Cuculiformes	Cuculidae	3	4	1	25%	2	50%	0	0%	1	25%	0	0%
Falconiformes	Accipitridae	2	2	1	50%	1	50%	0	0%	0	0%	0	0%
Galliformes	Numididae	1	1	0	0%	1	100%	0	0%	0	0%	0	0%
	Phasianidae	3	6	1	17%	1	17%	2	33%	3	50%	1	17%
Gruiformes	Rallidae	1	2	2	100%	0	0%	0	0%	0	0%	0	0%
Musophagiformes	Musophagidae	1	2	0	0%	1	50%	0	0%	0	0%	1	50%
Passeriformes	Alaudidae	1	3	2	67%	0	0%	0	0%	1	33%	0	0%
	Cisticolidae	11	45	27	60%	4	9%	8	18%	9	20%	5	11%
	Corvidae	1	3	1	33%	1	33%	1	33%	1	33%	0	0%
	Dicruridae	1	5	2	40%	1	20%	0	0%	1	20%	1	20%
	Emberizidae	2	2	1	50%	1	50%	0	0%	0	0%	0	0%
	Estrildidae	11	44	18	41%	5	11%	15	34%	13	30%	2	5%
	Eurylaimidae	1	1	0	0%	0	0%	1	100%	0	0%	0	0%
	Fringillidae	4	23	8	35%	6	26%	9	39%	4	17%	4	17%
	Hirundidae	3	5	3	60%	1	20%	1	20%	0	0%	0	0%
	Laniidae	1	1	0	0%	0	0%	0	0%	0	0%	1	100%
	Malaconotidae	6	15	12	80%	0	0%	6	40%	2	13%	2	13%
	Monarchidae	2	5	1	20%	1	20%	3	60%	0	0%	0	0%

	Motacillidae	1	2	1	50%	0	0%	0	0%	1	50%	0	0%
	Muscicapidae	12	62	27	44%	14	23%	24	39%	14	23%	7	11%
	Nectariniidae	8	28	6	21%	14	50%	6	21%	6	21%	1	4%
	Oriolidae	1	2	0	0%	1	50%	0	0%	0	0%	1	50%
	Paridae	2	2	2	100%	0	0%	1	50%	0	0%	1	50%
	Passeridae	3	7	3	43%	2	29%	1	14%	1	14%	0	0%
	Platysteiridae	2	20	8	40%	3	15%	8	40%	4	20%	5	25%
	Ploceidae	9	57	38	67%	4	7%	16	28%	5	9%	6	11%
	Pycnonotidae	5	44	12	27%	15	34%	28	64%	4	9%	7	16%
	Remizidae	1	1	0	0%	0	0%	0	0%	1	100%	0	0%
	Stenostiridae	1	1	0	0%	0	0%	0	0%	1	100%	0	0%
	Sturnidae	4	12	4	33%	7	58%	4	33%	2	17%	1	8%
	Sylviidae	9	33	8	24%	9	27%	6	18%	13	39%	2	6%
	Timaliidae	2	6	1	17%	1	17%	4	67%	0	0%	2	33%
	Turdidae	4	15	5	33%	3	20%	8	53%	3	20%	1	7%
	Viduidae	1	1	0	0%	0	0%	0	0%	1	100%	0	0%
	Zosteropidae	1	14	3	21%	13	93%	10	71%	0	0%	1	7%
Piciformes	Indicatoridae	3	5	2	40%	1	20%	3	60%	3	60%	0	0%
	Picidae	2	3	1	33%	1	33%	1	33%	1	33%	0	0%
	Ramphastidae	3	6	0	0%	3	50%	0	0%	2	33%	1	17%
Psittaciformes	Psittacidae	1	1	1	100%	0	0%	0	0%	0	0%	0	0%
Trogoniformes	Trogonidae	1	2	1	50%	1	50%	1	50%	0	0%	1	50%

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Parasitism by all three genera of haemosporidia generally increased with increasing nest height, excluding cliff/bank nesters (Figure 3.2 d–f). Nest location was the most important trait determining *Haemoproteus* infection rate, and was the second most important trait determining *Leucocytozoon* infection rate, based on the summed AIC-weights of the models containing these predictor variables. However, support for an effect of nest location on *Plasmodium* infection rates was relatively low compared to other variables (flocking behavior and nest type), based on this same criterion. Birds nesting in the canopy and sub-canopy were more likely than ground nesters to be infected by *Haemoproteus* or *Leucocytozoon*, and those nesting in the understory were also more likely to be infected by *Leucocytozoon* than ground-nesting birds. Birds nesting on cliffs or banks were more likely than ground nesters to be infected by *Plasmodium*, but support for this effect was comparatively weak (Table 3.5).

Flocking behavior was associated with parasitism rates by *Plasmodium* and *Haemoproteus*, but had no effect on *Leucocytozoon* parasitism rates. Birds in single-species flocks had lower rates of *Plasmodium* infection and higher rates of *Haemoproteus* infection, relative to solitary birds or birds living in mixed-species flocks (Figure 3.2 g-h). Inversely, habitat was the most important predictor affecting *Leucocytozoon* infection rates (Figure 3.2 i), but habitat had no effect on *Plasmodium* or *Haemoproteus* infection rates. The probability of infection by *Leucocytozoon* was highest in birds of evergreen forest habitat (n = 163; 26 host species), and was higher for birds of grassland/marsh (n = 114; 32 host species) and forest edge (n = 115; 40 species) habitats, relative to birds of woodland habitat (n = 61; 30 host species).

We found no host phylogenetic effect on rates of *Leucocytozoon* infection, but random effects in our models suggest that there may be additional, phylogenetically-constrained traits influencing parasitism rates at the host species level for *Haemoproteus*, and at the host family

level for *Plasmodium* (Table 3.3). We also found that the *Plasmodium* infection rate was higher and the *Leucocytozoon* infection rate lower at the dryer, low elevation site (Vwaza Marsh Wildlife Reserve), compared to infection rates at the wetter, higher elevation site (Nyika National Park). No effect of site existed for the rate of *Haemoproteus* infection.

Prevalence and diversity of haemosporidian parasite assemblages in Malawi

Of the 532 individual birds sampled in this study, 421 were infected by one or more haemosporidian parasite lineage. Prevalence among individual hosts was 48.2% for *Plasmodium*, 31.4% for *Haemoproteus* (subgenera. *Haemoproteus* and *Parahaemoproteus*), and 47% for *Leucocytozoon* respectively, with a total haemosporidian infection prevalence of 79.1% (Table 3.7). Of the infected individuals, 222 (52.7%) harbored coinfections. Of these co-infected individuals, 172 (40.9%) were infected by two or more identifiable parasite genera (Table 3.8). Of the remaining 50 co-infected individuals, 30 (11.8% of all coinfections) were infected by at least one *Leucocytozoon* parasite.

Plasmodium and *Leucocytozoon* coinfections occurred most frequently (n = 64 individuals; 15.2% of total infected), followed by *Haemoproteus* and *Leucocytozoon* coinfections (n = 48; 11.4%). Coinfection of a single host by congeneric parasites was rare, occurring most frequently with *Plasmodium* lineages (n = 28; 6.7%), about half as frequently for *Leucocytozoon* lineages (n = 16; 3.8%), and only once with *Haemoproteus* lineages (n = 1; 0.2%). Coinfection of individual hosts by all three parasite genera was also rare (n = 6; 1.4%).

Table 3.7. Haemosporidian abundance and diversity

	Plasmodium	*Haemoproteus*	*Leucocytozoon*	Total
Individuals infected (n)	203	132	198	421
% of total infected	48.2%	31.4%	47.0%	79.1%
Novel cytochrome *b* lineages	59	53	89	201
Described cytochrome *b* lineages[a]	22	16	9	47
Total lineages identified	81	69	98	248

[a]See MalAvi database for lineage information [57]

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Table 3.8. The distribution of resolved coinfections among three genera of parasites.

	P*P	H*H	L*L	P*H	P*L	H*L	P*H*L	TOTAL
Individuals (n)	28	1	16	10	64	48	6	172
% of total infected	6.7%	0.2%	3.8%	2.4%	15.2%	11.4%	1.4%	40.9%

P = *Plasmodium* spp.
H = *Haemoproteus* spp.
L = *Leucocytozoon* spp.

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From a total of 152 host species screened, we identified 248 unique haemosporidian mtDNA lineages, 201 (81%) of which have not been reported previously (Table 3.7), based on BLAST queries of *cytb* haplotypes against the MalAvi database (Grand Alignment, 27 August 2013 version; Bensch et al. 2009). New lineages documented in this study included 59 of 81 (72%) *Plasmodium* haplotypes, 53 of 69 (77%) *Haemoproteus* haplotypes, and 89 of 98 (91%) *Leucocytozoon* haplotypes.

Discussion

Relationships among haemosporidian infection rates and avian hosts' nesting traits in northern Malawi were consistent with previously hypothesized effects of host nesting biology on the transmission of vector-borne parasites (Blackmore et al. 1958; Caillouët et al. 2013; Edman and Kale 1971; Edman and Scott 1987; Kale et al. 1972; Scott and Edman 1991). Traits not directly related to nesting biology, such as flocking behavior and habitat, also appeared to have an effect on haemosporidian infection rates. Variation in the reliability of host traits to predict parasitism rates of the three haemosporidian parasite genera underscores the importance of vector biology in determining which host species are parasitized. The large number of novel parasite lineages we identified in our study suggests that either transmission to Palearctic migrant hosts occurs infrequently, that competent vectors of these novel African parasite lineages do not exist in the Palearctic, or that Palearctic migrant species wintering in Malawi nest in parts of the Palearctic that have not been well-surveyed for haemosporidians.

Association between life history traits and parasite prevalence

Our study is the first to examine the relationship between life history traits and haemosporidian parasitism across a broad taxonomic scale in the Afrotropics, where haemosporidian parasite diversity generally has been thought to be low compared to that in temperate regions (Valkiūnas 2005). We found that multiple bird life history traits, some of which are related directly to nesting biology, are significantly associated with rates of parasitism by haemosporidia in Malawi. However, these effects are qualitatively different among the three parasite genera, mirroring studies in temperate regions, which have found mixed support for the ability of ecological variables to predict haemosporidian prevalence (Bennett and Fallis 1960; Garvin and Remsen 1997; Read 1991; Ricklefs et al. 2005). Some have found a positive correlation between nest height and infection prevalence (Bennett and Fallis 1960; Garvin and Remsen 1997), whereas others have found no such correlation, even with comparable sampling from the same general temperate region (Ricklefs et al. 2005). With respect to nest type, Palearctic species with closed cup nests have a lower probability of infection by haemosporidian parasites, possibly because closed cup nests physically reduce the access of vectors (Valkiūnas 2005).

Several notable studies have considered the effects of environmental and ecological parameters on parasitism rates in focal Afrotropical bird species. For example, Sehgal et al. (2011) constructed spatial models using climatological and satellite-based habitat and topography data to predict the prevalence of *Plasmodium* and *Trypanosoma* spp. in the olive sunbird (*Cyanomitra olivacea*). Another Afrotropical study of the olive sunbird and yellow-

whiskered greenbul (*Andropadus latirostris*) found differences in parasite prevalence between disturbed and undisturbed habitat in Southern Cameroon (Chasar et al. 2009).

An increasing number of studies examining a broad range of host taxa and their parasites have been conducted recently in the Neotropics, where analyses of host life history traits and ecological parameters are comparable to those we have conducted in Malawi. Two studies conducted in Brazil by Fecchio et al. (2011, 2013) examined the relationships between life history traits and prevalence of two haemosporidian genera (*Plasmodium* and *Haemoproteus*). In the first of these two studies, Fecchio et al. (2011) analyzed microscopy-based parasite data from 17 host families (16 passerine and 1 non-passerine) and found correlations between parasite prevalence and host traits, including nest type, nest height, and flocking behavior. However, in a later molecular-based study by Fecchio et al. (2013), only flocking behavior was found to significantly explain rates of parasite prevalence among 17 families examined (12 passerine, 5 non-passerine). Another long-term Neotropical study in primary lowland forest used molecular methods to detect avian malaria infections in 22 passerine families, and found no correlation between *Plasmodium* or *Haemoproteus* prevalence and nest type (Svensson-Coelho et al. 2013). Most recently, a microscopy-based Colombian study spanning 12 years and 41 families (21 passerine, 19 non-passerine) examined the relationship between parasitism rates and avian life history traits including nest height, nest type, foraging height, flocking behavior, and migratory behavior, as well as environmental variables such as elevation, seasonality, and habitat (González et al. 2014). González et al. (2014) found that both open and closed cup nesting species experienced higher rates of parasitism by *Plasmodium*, whereas nest type had no effect on rates of parasitism by *Haemoproteus* or *Leucocotyzoon.* Species nesting at mid-understory height were more likely to be parasitized by *Haemoproteus*, but nest height had no effect on

Plasmodium or *Leucocytozoon* parasitism rates. Lastly, species that participated in mixed-species flocks experienced higher rates of parasitism by both *Haemoproteus* and *Leucocytozoon*, but flocking behavior had no effect on *Plasmodium* parasitism rates.

Our results from northern Malawi only partially match findings in the Neotropics. Similar to the results of Fecchio et al. (2011), but in contrast to those of Svensson-Coelho et al. (2013), we found that, in Malawi, birds building closed cup nests tend to have higher rates of *Plasmodium* infection and lower rates of *Haemoproteus* infection relative to birds building open cup nests. This pattern is consistent with the hypothesis that mosquitoes depend on kairomones (host-derived chemical cues), such as ammonia, 1-octen-3-ol, and CO_2 compounds (Logan et al. 2010), to locate hosts for bloodmeals. Host behaviors that increase the accumulation of such chemical compounds are more likely to attract mosquitoes, and therefore more likely to expose hosts to vector-borne parasites. The accumulation of kairomones in closed cup nests may therefore lead to an increased rate of contact between avian hosts and *Plasmodium*-transmitting mosquitoes (Withers et al. 1978). This hypothesis is further supported by reported positive correlations between nestling age, increasing metabolic mass (nestlings and adults), and CO_2 content within the nest (Wickler et al. 1980). On the other hand, the lower rate of parasitism by *Haemoproteus* in closed cup nesting birds may be due to the hypothesized dependence of biting midges (the definitive hosts of *Haemoproteus* parasites) on visual cues rather than olfactory cues. Although many vectors rely on a number of sensory stimuli, including olfactory, gustatory, and visual cues (Bernier et al. 1999; Bidlingmayer 1994; Khan 1977; Muir et al. 1992; Takken 1991), visual cues have been shown, both naturally and experimentally, to play a more important role than kairomones in the host-seeking responses of biting midges (Bishop 2002; Bishop et al. 2008). Additional support for the prioritization of visual over olfactory cues comes from the

observation that biting midge numbers are significantly reduced in outdoor areas that are covered, such as stables or sheds (Muller 1991), which Bishop (2002) attributes to the blocked vision of host-seeking midges.

Flocking behavior of birds from Malawi had significant and inverse associations with the probability of *Plasmodium* and *Haemoproteus* infections. Social species living in mixed- and single-species flocks have been reported to experience higher rates of *Haemoproteus* parasitism (Fecchio et al. 2011, 2013). However, in our study system we did not find a strong relationship between higher infection rates and species' sociality per se. Instead, we found that bird species aggregating in single-species flocks experienced a lower probability of infection by *Plasmodium* parasites and a higher probability of infection by *Haemoproteus* parasites, relative to birds that are solitary or living in mixed-species flocks. This result is the opposite of that found in Colombian birds by González et al. (2014), who showed that birds of mixed-species flocks experienced higher rates of parasitism by *Haemoproteus.* A recent study by Janousek et al. (2014) suggests that communal roosting in single-species flocks might be influenced by pathogen-mediated selection, driven by host–vector ratios and encounter rates of birds and virus-transmitting *Culex* mosquitoes (Janousek et al. 2014). Similar mechanisms may influence rates of parasitism by *Plasmodium* parasites, which are also vectored by *Culex* mosquitoes, and could explain the lower rates of infection by *Plasmodium* parasites that we observed among birds aggregating in single-species flocks, particularly given that bird species in this category also tend to roost communally (e.g., queleas, widowbirds, starlings, weavers, etc.). It is unclear why rates of infection by *Haemoproteus* parasites for birds in single-species flocks are increased. This phenomenon may be related to the behavior and host specificity of vectors. The inverse effects of flocking behavior on *Plasmodium* versus *Haemoproteus* infection rates suggest that the

respective vectors of these malaria parasites, the parasites themselves, or some combination thereof, respond differently to the species composition in groups of aggregating hosts.

Similar to Fecchio et al. (2011), we found higher parasitism rates with increasing average nest height. This correlation is consistent with the observations of Bennett and Fallis (1960) from the Nearctic, that vector abundance is vertically stratified, and thus birds nesting at higher strata should experience increased rates of parasitism due to increased contact with vectors. Although this hypothesis has not been tested for ornithophilic vectors in the east African tropics, our results suggest that mechanisms similar to those in the Nearctic may also influence host–vector contact rates in the Afrotropics.

The effect of habitat on parasitism rates can vary from region to region, and may be influenced by many factors in the study design, including the number of taxa sampled, number of habitats compared, and size of the study site/region (Fecchio et al. 2011; Lacorte et al. 2013; Loiseau et al 2010;). We found no effects of habitat on *Plasmodium* or *Haemoproteus* infection rates. However, rate of *Leucocytozoon* infection is strongly affected by habitat. Specifically, when compared to birds from all other habitats sampled (excluding aquatic habitat due to inadequate sample sizes), those living in evergreen forest experienced the highest rates of *Leucocytozoon* infection, whereas birds from woodland habitats experienced the lowest rates of *Leucocytozoon* infection. This effect is likely caused by elements within these habitats that are related to vector ecology, and the same is possibly true for life history or habitat effects on rates of parasitism by *Plasmodium* and *Haemoproteus*. *Plasmodium*, *Haemoproteus,* and *Leucocytozoon* are vectored by different dipteran insects, therefore aspects of vector feeding behaviors must be considered when attempting to discern the underlying causes of variation in rates of parasitism. More research is certainly needed in this area.

Prevalence, diversity, and geographic range of avian Plasmodium, Haemoproteus, and Leucocytozoon lineages found in Malawi

Total prevalence of haemosporidian parasites in our Malawi samples is high (78.1%) relative to studies of avian haemosporidian prevalence with comparable numbers of host species from other geographic regions (Table 3.9). If only considering *Plasmodium* and *Haemoproteus*, the prevalence from our Malawi study is 66.6% (43.9% and 25.8% respectively, with many individual hosts harboring coinfections). Ricklefs et al. (2005) proposed that with sufficient sampling, the number of malaria parasite lineages within a local geographic area should approximate the number of host species. Our data support this hypothesis, finding 149 malaria parasite lineages (*Plasmodium* spp. and *Haemoproteus* spp.) in the 152 bird species sampled, far exceeding the malaria parasite diversity previously described for this region by Loiseau et al. (2011). Our results also suggest that the Afrotropics harbor greater prevalence and genetic diversity of *Leucocytozoon* parasites than previously found in any other region of the world (Table 3.9).

Table 3.9. Comparative overview of some studies in avian haemosporidian prevalence and diversity

Location	Study length	Host Ord. (n)	Host Fam. (n)	Host Spec. (n)	Individ. Sampled (n)	%*P*	%*H*	%*L*	Total prevalence among all individuals sampled*	Parasite detection method	Parasite lineages identified (n)	References
AFRICA												
Malawi	2009	16	50	152	532	50	23.6	36.1	79.1%	PCR	248	This study
Cameroon Gabon	2002 – 2004	9	29	93	527	45	23	7	NA	PCR	117	Beadell et al. 2009
Madagascar	1993 – 2004	7	26	64	947	1.9	17.4	9.4	NA	Micro-scopy	45	Savage et al. 2009
Cameroon Gabon Tanzania Malawi South Africa	NA	1	8	25	1364	20.1	NA	NA	20.1%	PCR	34	Loiseau et al. 2011
Western Indian Ocean	1999 – 2002	1	6	21	150	32	13.3	NA	45.3%	PCR	16	Ishtiaq et al. 2012
ASIA												
Myanmar	1994 – 2004	5	52	133	335	20.3	13.4	NA	34%	PCR & Micro-scopy	75	Ishtiaq et al. 2007
India				43	183	27.9	18	NA				
South Korea				46	181	30.9	11	NA				
AUSTRALIA												
Australia	2002 – 2003	3	8	32	219	14	28	NA	44%	PCR	78	Beadell et al. 2004
Papua NewGuinea	1991 – 2002			77	209	10	31	NA				
New Zealand	2003 – 2006	3	21	25	820	52.9	NA	NA	52.9%		8	Ewen et al. 2012

NORTH AMERICA												
United States Canada	~1940 – 1975	17	55	388	57026	3.8	19.5	17.7	NA	Micro-scopy	NA	Greiner et al. 1975
Costa Rica	1987 – 1991	4	15	60	479	0.4	9.4	0.4	11%	Micro-scopy	NA	Young et al. 1993
United States	1988	1	8	19	935	3.4	22.8	1.3	NA	Micro-scopy	NA	Garvin & Remsen 1997
Lesser Antilles	NA	7	17	53	1975	10.3	36.1†	NA	27.6%	PCR & Micro-scopy	26	Fallon et al. 2005
United States	1999 – 2002	2	13	42	757	NA	NA	0	~38.6%	PCR & Micro-scopy	34	Ricklefs et al. 2005
SOUTH AMERICA												
Panama	1969 – 1976	4	36	281	3715	5	9	< 1	~15%	Micro-scopy	NA	Sousa & Herman 1982
Bolivia	1988	7	25	135	641	1.1	1.4	NA	2.5%	Micro-scopy	NA	Bennett et al.1991
Brazil	2000	1	9	45	275	39.6	NA	NA	39.6%	PCR & Micro-scopy	NA	Ribeiro et al. 2004
Guyana	1994 – 2000	4	10	53	195	24.6	13.8	NA	42.1%	PCR & Micro-scopy	59	Durrant et al. 2006
Uruguay	2002 – 2003		41	111	322	17.8	3.7	NA	24.2%			
Colombia	2001 – 2002	4	12	75	302	5.6	2.6	0.3	8.6%	Micro-scopy	NA	Londoño et al. 2007
Chile	2003 – 2005	NA	NA	26	617	6.5	5.0	8.9	15.4%	PCR & Micro-scopy	27	Merino et al. 2008

Colombia	2002 – 2003	5	14	40	136	8.1	1.5	21.3	NA	Micro-scopy	NA	Rodríguez et al. 2009
Brazil	2007 – 2009	2	29	122	676	49 combined		NA	49%	PCR & Micro-scopy	21	Belo et al. 2011
Brazil	2005 – 2009	2	6	17	772	3.6	7.1	0	10.7%	Micro-scopy	NA	Fecchio et al. 2011
Venezuela	2011	2	12	24	47	4.3	6.4	2.1	10.6%	PCR	6	Mijares et al. 2012
Brazil	2005 – 2009	6	18	54	790	4.8	11.3	NA	16.1%	PCR	22	Fecchio et al. 2013
Brazil	2000 – 2006 2010	2	21	194	1545	23	4.5	NA	35.3%	PCR	110	Lacorte et al. 2013
Ecuador	2001 – 2010	1	22	144	2488	9	6	NA	21.7%	PCR	65	Svensson-Coelho et al. 2013
Colombia	1999 – 2011	NA	41	169	246	3.0	5.0	5.0	12.8%	Micro-scopy	NA	González et al. 2014

*Calculations based on total individuals infected out of total individuals sampled N_i/N; values are not always directly comparable between studies due to variation in haemosporidian genera examined.

†Lineages n = 3 found in doves but not assigned to haemosporidian genus were considered *Haemoproteus* Haemoproteus spp. and included in the calculation of % of *Haemoproteus* infection.

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Our findings underscore the importance of broad host taxonomic sampling, as well as stringent detection methods. We attribute the high prevalence of infection detected in our study, in part, to the application of multiple PCR screens, which frequently detected new unique parasite lineages in each round of PCR. In another recent PCR-based study, Lacorte et al. (2013) examined haemosporidian diversity in 200 Brazilian bird species from 21 families (predominantly Passeriformes). Of the 89 *Plasmodium* and 22 *Haemoproteus* lineages identified by Lacorte et al. (2013), 86% constituted new lineages. Similar to our study, Lacorte et al. (2013) also found relatively high parasite lineage diversity among a large number of host species sampled, although diversity of malaria parasites from Malawian birds was considerably higher.

The majority of parasite lineages we identified in Malawi (n = 201; 81% of total identified) have not been detected previously, and therefore have not been recorded in other geographic regions. Of those lineages we identified that have been described (n = 47), 25 have been found only in Africa, 10 in both Africa and the western Palearctic, 5 only in the western Palearctic, 3 globally, 2 in Asia, 1 in both Africa and the Middle East, and 1 in North America (Fig 3.2 a-c; also see Appendix E; MalAvi database). The high occurrence of novel parasite lineages in our study may indicate endemic parasite species and it also suggests that avian Palearctic migrants are not suitable hosts for a number of malaria parasite lineages with which they geographically overlap during their wintering season in Africa. However, we cannot rule out the possibility that the absence of competent vectors and/or poor sampling in Palearctic regions (e.g., Western Asia) explains this pattern. We speculate that the high parasite diversity we observed, relative to other studies in Africa (e.g., Beadell et al. 2009; Loiseau et al. 2010; 2011), might be a consequence of Malawi's biogeographic position as a meeting place of avian taxa from the south, central, and eastern regions of Africa (Dowsett-Lemaire and Dowsett 2006).

More data from neighboring regions will help resolve this question. In general, our findings are consistent with those of Lacorte et al. (2013) and Clark et al. (2014), suggesting that haemosporidian parasite diversity is much higher in both the New and Old World tropics than previous surveys (Valkiūnas 2005) concluded on the basis of microscopy.

Conclusion

We report new avian haemosporidian data from a broad survey of birds from southeast Africa, a relatively underrepresented region in the history of avian haemosporidian research. Using sensitive molecular methods and multiple screenings to overcome potentially low parasitemia levels in our samples, we found unprecedented prevalence of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* infections across a broad range of birds belonging to 16 orders, 50 families, 100 genera, and 152 species. Our analyses of avian host life history traits revealed a diversity of relationships between life history characteristics and haemosporidian infection rates for three different parasite genera. The positive correlation between nest height and probability of haemosporidian infection appears to be a consistent pattern across geographic regions. Similar to Neotropical studies, we found differing effects of nest type for each haemosporidian parasite genus. Birds with closed cup nests experienced increased rates of *Plasmodium* infection and decreased rates of *Haemoproteus* infection, whereas cavity-nesting birds experienced increased rates of *Leucocytozoon* infection. The effects of these host traits on haemosporidian infection rates have never been studied in the African tropics, and our data demonstrate that this system contains higher parasite diversity and is equally complex, if not more so, than Neotropical and temperate systems. Haemosporidian prevalence in birds from our

Malawi study is higher than that found in other tropical regions, and we identify a relatively large number of new parasite lineages (n = 201; 81% of total identified). These results suggest that, as is the case with many other vector-borne pathogens, the Afrotropics is an area of high haemosporidian endemicity and diversity. The high prevalence, diversity, and possible endemicity of haemosporidians in this southeastern African region, as well as a presumably diverse array of dipteran vectors on which they rely for transmission, indicate their suitability as a model for research on vector-borne pathogens. Southeastern Africa is undeniably an important region in which to investigate the mechanisms underlying host–parasite associations, speciation, and the evolution of malaria parasites and other closely related haemosporidians.

ACKNOWLEDGEMENTS

We thank the Malawi Department of National Parks and Wildlife, and especially the management and staff of Vwaza Marsh Wildlife Reserve and Nyika National Park, for logistical and other support. We worked under permits from the Department of Forestry (License No. 3/12/2007/1, granted on 10 July 2009, valid from 10 July 2009 to 10 July 2010) and the Department of National Parks and Wildlife (Ref. No. NPW/2/1/12, granted on 6 October 2009, valid from October 2009 to November 2009). This survey was sponsored by the Field Museum's Emerging Pathogens Project, funded by the Davee Foundation and the Dr. Ralph and Mariam Falk Medical Research Trust. Staff of the Museums of Malawi, including Potiphar Kaliba, Christopher Salema, and Nyson Gawani, participated in the fieldwork and preparation of specimens. We thank Jacob Mertes for his help screening samples for *Leucocytozoon*. We also thank André Dhondt and John Fitzpatrick for their helpful comments, which improved this manuscript. DNA sequencing for this project was carried out in the Field Museum's Pritzker Laboratory for Molecular Systematics and Evolution, operated with support of the Pritzker Foundation.

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CHAPTER 4

DIVERSE SAMPLING OF EAST AFRICAN HAEMOSPORIDIANS REVEALS CHIROPTERAN ORIGIN OF MALARIA PARASITES IN PRIMATES AND RODENTS[3]

Abstract

Phylogenies of parasites provide hypotheses on the history of their movements between hosts, leading to important insights regarding the processes of host-switching that underlie modern-day epidemics. Haemosporidian (malaria) parasites lack a well-resolved phylogeny, which has impeded the study of evolutionary processes associated with host-switching in this group. Here, we present a novel phylogenetic hypothesis that suggests that bats served as the ancestral hosts of malaria parasites in primates and rodents. Expanding upon current taxon sampling of Afrotropical bat and bird parasites, we find strong support for all major nodes in the haemosporidian tree using both Bayesian and maximum likelihood approaches. Our analyses support a single transition of haemosporidian parasites from saurian to chiropteran hosts, and do not support a monophyletic relationship between *Plasmodium* parasites of birds and mammals. We find, for the first time, that *Hepatocystis* and *Plasmodium* parasites of mammals represent reciprocally monophyletic evolutionary lineages. These results highlight the importance of broad taxonomic sampling when analyzing phylogenetic relationships, and have important implications for our understanding of key host-switching events in the history of malaria parasite evolution.

[3] Lutz HL, Patterson BD, Kerbis JC, Webala PW, Stanley WT, Gnoske TP, Hackett SJ, Stanhope MJ. 2016. Diverse sampling of East African haemosporidians reveals chiropteran origin of malaria parasites in primates and rodents. Mol Phylogenet Evol. 99: 7 – 15.

Introduction

Phylogenies are an important tool in the study of zoonotic epidemics, helping to determine the source of a pathogen or parasite, and enabling the prediction of future outbreaks by identifying evolutionary lineages with significant host-switching potential (Suzán et al, 2015). The utility of a phylogeny depends on adequate genetic and taxon sampling, and is limited by these two variables (Nabhan and Sarkar, 2012). Assumptions about evolutionary variables are often a necessary component of phylogenetic models, and these assumptions too can limit the accuracy of a phylogeny. Common problems facing phylogenetic analyses, therefore, are typically linked to sparse taxon sampling, gene sampling, or inaccurate evolutionary assumptions regarding outgroup assignment or rates of evolutionary change across disparate phylogenetic lineages.

Such problems have hindered phylogenetic analyses of malaria parasites (Apicomplexa: Haemosporida), which are one of the most diverse assemblages of protozoan parasites, including species that cause epidemic diseases in humans. In addition to sparse or biased taxon sampling, a lack of well-developed phylogenetic markers, and uncertainty regarding the root of the haemosporidian phylogeny, malaria systematists have also been hindered by the ambiguity of morphological features and complex life histories used to distinguish species in this group (Valkiūnas 2005). Indeed, the evolutionary origin of the deadliest human parasite, *Plasmodium falciparum*, remains unresolved. Lateral transfer of a malaria parasite from birds to humans was once implicated in the high pathogenicity exhibited by *P. falciparum*, and was supported by early molecular studies (McCutchan et al. 1996; Waters et al. 1991). However, the discovery of a reservoir of diverse, closely-related parasites in non-human primates, and a plethora of studies in

recent decades (e.g., Martinsen et al. 2008a; Outlaw and Ricklefs 2011; Perkins and Schall 2002), have since rendered this avian origin hypothesis obsolete (Duval et al. 2010; Krief et al. 2010; Ollomo et al. 2009; Prugnolle et al. 2010).

The discovery of new malaria parasites in African primates has altered our understanding of how *P. falciparum* may have arisen in humans, and has underscored the importance of thorough sampling when assessing the origin of epidemic pathogens or parasites. Major efforts to document haemosporidians in wild hosts have improved our knowledge of genetic diversity within the group (Beadell et al. 2009; Falk et al. 2011; Fecchio et al. 2013; Lacorte et al. 2013; Lutz et al. 2015; Svensson-Coelho et al. 2013), but many hosts and geographic regions remain largely unexplored. For instance, the Old World tropics contain some of the highest levels of vertebrate species richness (Davies and Buckley 2011; Jan Schipper et al. 2008; Jetz and Rahbek 2001; Jetz et al. 2012), yet few broadscale surveys of Afrotropical or Asian haemosporidians have been conducted. Even fewer systematic studies in these regions have included both saurian (bird and reptile) and mammalian parasites in their phylogenetic analyses (Duval et al. 2012; Schaer et al. 2013). Parasites of Afrotropical bats are of particular interest due to their diversity and ambiguous positions in the haemosporidian Tree of Life. Molecular surveys of chiropteran haemosporidians have identified new species with the potential to inform human malaria research (Schaer et al. 2013) and have suggested that improved sampling of chiropteran parasites may clarify the evolutionary origins of haemosporidians in other groups (Duval et al. 2007; Duval et al. 2012; Schaer et al. 2013; Witsenburg et al. 2012).

A major question is whether mammalian parasites form a clade, or whether chiropteran haemosporidians represent a secondary invasion of mammals by a parasite from a non-mammalian host – a hypothesis posited by several recent studies (Duval et al 2012; Megali et al.,

2011; Outlaw and Ricklefs 2011; Witsenburg et al. 2012). Recent work employing multiple nuclear markers from both avian and mammalian parasites found support for a monophyletic relationship of all *Plasmodium* species, rendering mammalian haemosporidians a paraphyletic group. The same study found strong support for the assignment of *Leucocytozoon* as the outgroup to all other haemosporidians (Borner et al. 2016). Such discoveries have important implications for how life history traits, such as erythrocytic schizogony, may have evolved. In light of such discoveries and in the presence of new data from haemosporidians of disparate hosts and geographic regions, a re-evaluation of the phylogenetic history of malaria parasites is therefore both important and timely (Perkins 2014; Rich and Xu 2011).

In this study, we present a novel phylogenetic hypothesis for the haemosporidian Tree of Life, based on improved sampling of avian and mammalian hosts. Taxon sampling included the first large-scale systematic survey of neglected chiropteran parasites in the East African tropics from a range of habitats in Kenya, Malawi, Mozambique, Tanzania, and Uganda. We paired these data with comparable sampling of avian parasites, and sequenced genes from each of the three genomes present in malaria parasites (nuclear, mitochondrial, and apicoplast). Combining these new data with a broad representation of parasites from reptiles, humans, non-human primates (including novel primate parasites in the *Laverania* subgenus), and additional birds and bats, we re-evaluated major evolutionary relationships in the malaria Tree of Life. We explicitly tested the hypothesis that *Plasmodium* parasites from birds and mammals are monophyletic, while reconsidering the evolutionary relationships between chiropteran and non-chiropteran parasites of mammals. Support for our phylogenetic hypothesis suggests that bats, not birds or reptiles, were the ancestral hosts of extant *Plasmodium* parasites in mammals. Our results align well with previous studies, revealing that host-switching of parasites from bats to other

vertebrates appears to be common throughout the haemosporidian phylogeny. This novel phylogenetic hypothesis has important implications for inferences regarding trait evolution and host shifts of haemosporidian parasites between vertebrate classes, as well as shifts between invertebrate vectors.

Methods

Sampling

We sampled 791 mammals, including 505 bats and 286 rodents and shrews (Appendix G, H). Bats were sampled from both suborders of Chiroptera (Yinpterochiroptera and Yangochiroptera), representing 46 species from 8 families (Table 4.1). Sampling was conducted between 2009 and 2014, at sites in Kenya, Malawi, Mozambique, Tanzania, and Uganda (Figure 4.1; Appendix I). Bats were captured using mist-nets, triple-high mist nets, harp traps, or hand nets (at roosts). In addition to bats, rodents and shrews from sites in Malawi, Mozambique, and Uganda were collected using a combination of Sherman, pitfall, and snap traps. Bird sampling was conducted concurrently at all sites, except for those in Kenya and Tanzania, according to previously described methods (Lutz et al. 2015), and included 1,745 individuals representing 20 avian orders and 112 families (> 400 species) (bird data available via the Field Museum of Natural History Bird Collection Database, fm1.fieldmuseum.org/birds/). Blood was stored on Whatman Classic FTA cards, and thin blood films were prepared when possible. All sampling was conducted in accordance with the Field Museum of Natural History IACUC, and voucher specimens of both mammals and birds are accessioned at the Field Museum of Natural History.

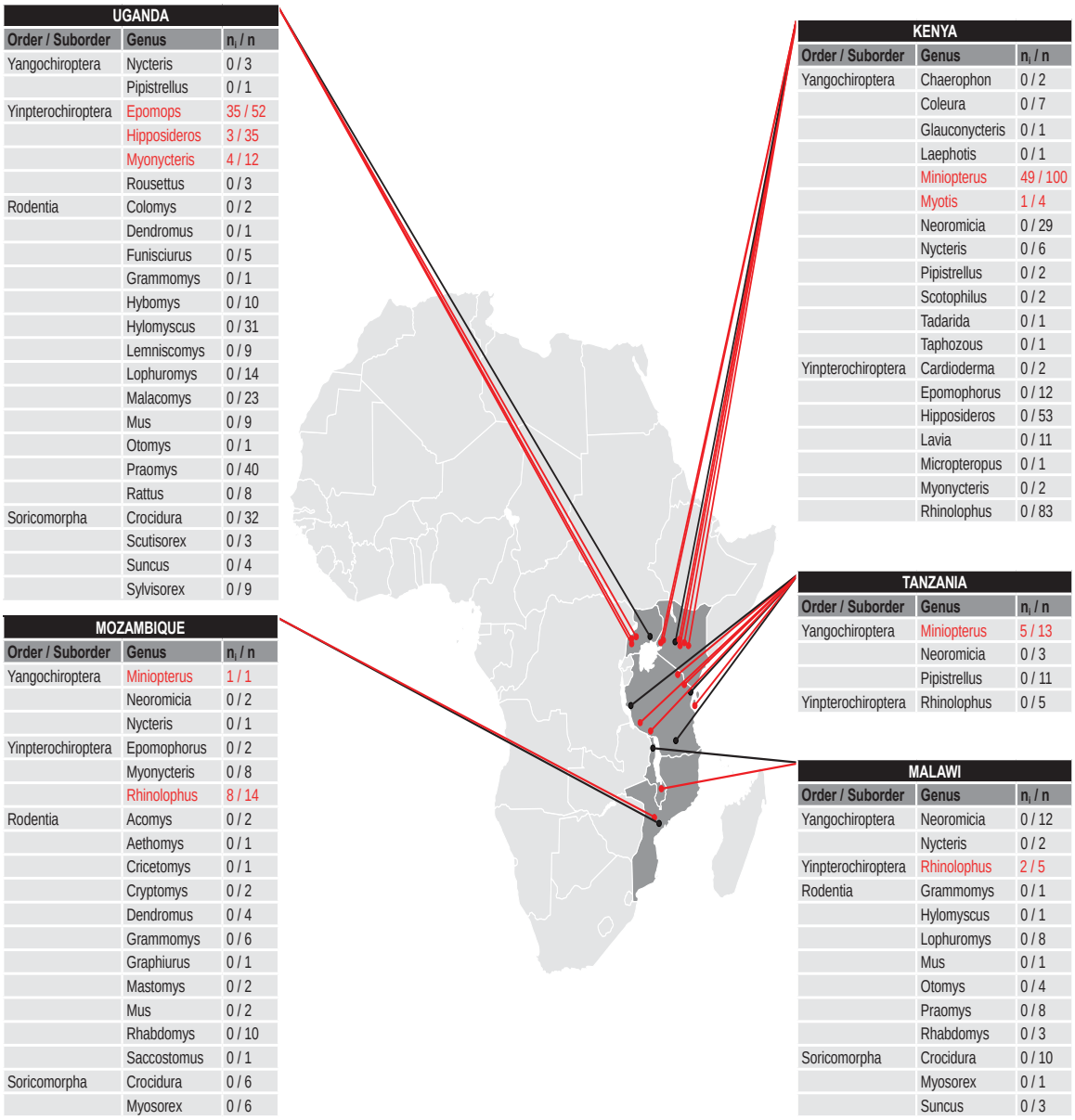
Table 4.1. Taxonomic sampling of bats from East Africa, with number of individuals sampled (n) and infected (n_i).

Host Family	Host Species	n_i	n	Parasite
YANGOCHIROPTERA				
Emballonuridae	*Coleura afra*	0	7	
Emballonuridae	*Taphozous mauritianus*	0	1	
Miniopteridae	***Miniopterus africanus***	**4**	**20**	***Polychromophilus melanipherus***
Miniopteridae	***Miniopterus cf. fraterculus***	**2**	**6**	***Polychromophilus melanipherus***
Miniopteridae	***Miniopterus minor***	**5**	**13**	***Polychromophilus melanipherus***
Miniopteridae	***Miniopterus natalensis***	**20**	**42**	***Polychromophilus melanipherus***
Miniopteridae	***Miniopterus rufus***	**22**	**31**	***Polychromophilus melanipherus***
Miniopteridae	***Miniopterus* sp.**	**2**	**2**	***Polychromophilus melanipherus***
Molossidae	*Chaerophon pumilus*	0	2	
Molossidae	*Tadarida cf. lobata*	0	1	
Nycteridae	*Nycteris arge*	0	1	
Nycteridae	*Nycteris aurita*	0	1	
Nycteridae	*Nycteris macrotis*	0	1	
Nycteridae	*Nycteris* sp.	0	2	
Nycteridae	*Nycteris thebaica*	0	7	
Vespertilionidae	*Glauconycteris humeralis*	0	1	
Vespertilionidae	*Laephotis wintoni*	0	1	
Vespertilionidae	*Myotis bocagii*	0	1	
Vespertilionidae	***Myotis tricolor***	**1**	**3**	***Polychromophilus* sp.**
Vespertilionidae	*Neoromicia capensis*	0	16	
Vespertilionidae	*Neoromicia nana*	0	16	
Vespertilionidae	*Neoromicia* sp.	0	6	
Vespertilionidae	*Neoromicia tenuipinnis*	0	8	
Vespertilionidae	*Pipistrellus hesperidus fuscatus*	0	11	
Vespertilionidae	*Pipistrellus rueppellii*	0	2	
Vespertilionidae	*Pipistrellus* sp.	0	1	
Vespertilionidae	*Scotophilus dinganii*	0	1	
Vespertilionidae	*Scotophilus* cf. *leucogaster*	0	1	
YINPTEROCHIROPTERA				
Hipposideridae	*Hipposideros beatus*	0	3	
Hipposideridae	*Hipposideros caffer*	0	38	
Hipposideridae	***Hipposideros cyclops***	**3**	**3**	***Nycteria* sp.**
Hipposideridae	*Hipposideros ruber*	0	43	
Hipposideridae	*Hipposideros* sp.	0	1	
Megadermatidae	*Cardioderma cor*	0	2	
Megadermatidae	*Lavia frons*	0	11	

Pteropodidae	*Epomophorous labiatus*	0	6	
Pteropodidae	*Epomophorus wahlbergi*	0	8	
Pteropodidae	***Epomops franqueti***	**35**	**52**	***Hepatocystis* sp.**
Pteropodidae	*Micropteropus pusillus*	0	1	
Pteropodidae	*Myonycteris angolensis*	0	15	
Pteropodidae	***Myonycteris torquata***	**4**	**7**	***Hepatocystis sp.***
Pteropodidae	*Rousettus aegyptiacus*	0	3	
Rhinolophidae	*Rhinolophus clivosus*	0	9	
Rhinolophidae	*Rhinolophus deckeni*	0	2	
Rhinolophidae	*Rhinolophus eloquens*	0	39	
Rhinolophidae	***Rhinolophus fumigatus***	**2**	**5**	***Nycteria* sp.**
Rhinolophidae	***Rhinolophus hildebrandti***	**8**	**14**	***Nycteria* sp.**
Rhinolophidae	*Rhinolophus landeri*	0	36	
Rhinolophidae	*Rhinolophus simulator*	0	1	
Rhinolophidae	*Rhinolophus* sp.	0	1	
TOTAL		108	505	

Identification and sequencing of haemosporidian parasites

Genomic DNA was extracted from whole blood that was stored on Whatman FTA Classic Cards, using the dried blood spot protocol of Qiagen Blood and Tissue Mini Kits (Qiagen, Valencia, CA). A polymerase chain reaction (PCR) protocol targeting a standard 478 bp barcoding region of the haemosporidian mitochondrial cytochrome *b* (*cytb*) gene (Bensch et al. 2009a) was applied to all DNA samples in triplicate (*sensu* Lutz et al. 2015). Thin blood films prepared from fresh blood were fixed in the field with 100% methanol and subsequently stained with a 10% Giemsa solution in the lab. Blood smears from individuals detected to be positive for



UGANDA

Order / Suborder	Genus	n_i / n
Yangochiroptera	Nycteris	0 / 3
	Pipistrellus	0 / 1
Yinpterochiroptera	Epomops	35 / 52
	Hipposideros	3 / 35
	Myonycteris	4 / 12
	Rousettus	0 / 3
Rodentia	Colomys	0 / 2
	Dendromus	0 / 1
	Funisciurus	0 / 5
	Grammomys	0 / 1
	Hybomys	0 / 10
	Hylomyscus	0 / 31
	Lemniscomys	0 / 9
	Lophuromys	0 / 14
	Malacomys	0 / 23
	Mus	0 / 9
	Otomys	0 / 1
	Praomys	0 / 40
	Rattus	0 / 8
Soricomorpha	Crocidura	0 / 32
	Scutisorex	0 / 3
	Suncus	0 / 4
	Sylvisorex	0 / 9

MOZAMBIQUE

Order / Suborder	Genus	n_i / n
Yangochiroptera	Miniopterus	1 / 1
	Neoromicia	0 / 2
	Nycteris	0 / 1
Yinpterochiroptera	Epomophorus	0 / 2
	Myonycteris	0 / 8
	Rhinolophus	8 / 14
Rodentia	Acomys	0 / 2
	Aethomys	0 / 1
	Cricetomys	0 / 1
	Cryptomys	0 / 2
	Dendromus	0 / 4
	Grammomys	0 / 6
	Graphiurus	0 / 1
	Mastomys	0 / 2
	Mus	0 / 2
	Rhabdomys	0 / 10
	Saccostomus	0 / 1
Soricomorpha	Crocidura	0 / 6
	Myosorex	0 / 6

KENYA

Order / Suborder	Genus	n_i / n
Yangochiroptera	Chaerophon	0 / 2
	Coleura	0 / 7
	Glauconycteris	0 / 1
	Laephotis	0 / 1
	Miniopterus	49 / 100
	Myotis	1 / 4
	Neoromicia	0 / 29
	Nycteris	0 / 6
	Pipistrellus	0 / 2
	Scotophilus	0 / 2
	Tadarida	0 / 1
	Taphozous	0 / 1
Yinpterochiroptera	Cardioderma	0 / 2
	Epomophorus	0 / 12
	Hipposideros	0 / 53
	Lavia	0 / 11
	Micropteropus	0 / 1
	Myonycteris	0 / 2
	Rhinolophus	0 / 83

TANZANIA

Order / Suborder	Genus	n_i / n
Yangochiroptera	Miniopterus	5 / 13
	Neoromicia	0 / 3
	Pipistrellus	0 / 11
Yinpterochiroptera	Rhinolophus	0 / 5

MALAWI

Order / Suborder	Genus	n_i / n
Yangochiroptera	Neoromicia	0 / 12
	Nycteris	0 / 2
Yinpterochiroptera	Rhinolophus	2 / 5
Rodentia	Grammomys	0 / 1
	Hylomyscus	0 / 1
	Lophuromys	0 / 8
	Mus	0 / 1
	Otomys	0 / 4
	Praomys	0 / 8
	Rhabdomys	0 / 3
Soricomorpha	Crocidura	0 / 10
	Myosorex	0 / 1
	Suncus	0 / 3

Figure 4.1. Map of sampling localities, and mammal genera sampled by country. Red text and red lines indicate genera and localities from which haemosporidian parasites were recovered.

haemosporidians by PCR were screened under 1000x magnification for 100 fields for visual confirmation of infection and morphological identification of parasites when possible (Figure 4.2). Blood films from rodents, shrews, and bats that screened negative by PCR were also

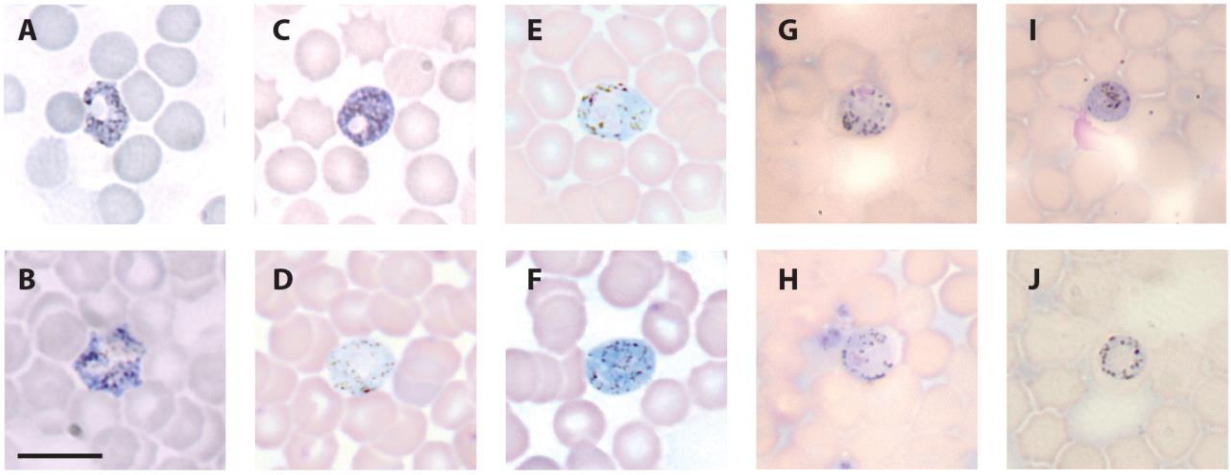


Figure 4.2. Micrographs of blood stage gametocytes of bat haemosporidians, 1000x magnification. (A – C) Hepatocystis cf. epomophori macrogametocytes ex. Epomops franquetti; (D – E) Polychromophilus melanipherus microgametocytes and (F) macrogametocyte ex. Miniopterus natalensis; (G – H) Nycteria microgametocyte, (I) macrogametocyte, and (J) gametocyte ex. Hipposideros cyclops. Scale bar = 10 µm.

examined to verify absence of haemosporidian parasites when possible (blood films were not available for all individuals).

Following the detection of parasites, PCR products from barcoding screens were purified and sequenced on an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, California). We then grouped sequences into unique lineages using the FaBox haplotype collapser (Villesen 2007), with unique lineages being defined as having one or more SNPs at the *cytb* locus. Due to the vast number of unique parasite lineages identified in the birds we sampled, data from all countries were included in preliminary phylogenetic analyses to provide phylogenetic context for the selection of a subset of lineages, and ultimately parasites from Malawi and Mozambique were included in this study (Appendix H). For additional sequencing and phylogenetic analyses, we selected a subset of these collapsed parasite lineages (which included representatives from *Plasmodium*, *Haemoproteus*, *Parahaemoproteus*, *Leucocytozoon*, *Polychromophilus*, *Nycteria*, and *Hepatocystis*) such that broad and even coverage of genetic diversity was obtained. For these parasite lineages, we sequenced additional loci from each of the three genomes present in Haemosporida, using the methods of Martinsen et al. 2008b:

mitochondrial cytochrome oxidase I (998 bp, trimmed to 886 for phylogenetic analyses), nuclear adenylosuccinate lyase (206 bp), and the apicoplast Caseinolytic protease C (531 bp). All sequences are accessioned on GenBank (KT750341 – KT750753) (Appendix J), and all avian *cytb* sequences are also available via the MalAvi database (Bensch et al., 2009b) (http://mbio-serv2.mbioekol.lu.se/Malavi/; Accessed 1 Feb 2015).

Phylogenetic analysis

Phylogenetic analyses were performed on a concatenated DNA sequence alignment of four genes (*cytb*, CoI, Asl, ClpC; 2536 bp) via the CIPRES Science Gateway Web Portal V3.3 (Miller et al. 2010) and the Computational Biology Service Unit at Cornell university (using a large memory machine with 512GB RAM and 64 cores). Our alignment consisted of chiropteran and avian parasite sequences from this study, combined with data available from GenBank representing additional parasites of bats, birds, primates, and squamate reptiles for a total of 170 unique parasite lineages (Appendix J). Sequences were aligned using the MUSCLE plugin for Geneious v7.1.7 (Biomatters Ltd.), and the best partitioning scheme and model of evolution were determined using PartitionFinder (Lanfear et al. 2012). We compared four partitioning schemes (no partition, partitioned by gene, partitioned by codon, and mtDNA combined versus nuDNA and apDNA) and found no well-supported differences in the resulting maximum likelihood tree topologies. A concatenated unpartitioned dataset and GTR+I+G model of evolution were used for subsequent phylogenetic analyses.

Bayesian inference (BI) analyses were implemented using BEAST v1.8.0 and its associated utilities (Drummond et al. 2012). We assigned *Leucocytozoon* as the outgroup, based

on the conclusions of Borner et al. (2016) regarding proper root assignment for Haemosporida. Following preliminary runs to optimize chain length and ensure proper mixing and convergence, we conducted four independent runs consisting of 150,000,000 generations, each using an uncorrelated relaxed lognormal clock and a Yule speciation prior. Appropriate mixing and convergence of runs, and effective sample size (ESS > 1000 in all cases, ESS > 2000 in most), were assessed in the program Tracer v1.6 (Rambaut et al. 2014). After the removal of burn-in (10%), runs were combined using the ancillary BEAST program, LogCombiner v1.8.0, and a maximum clade credibility tree was produced using the program TreeAnnotator v1.8.0 (Appendix K).

Maximum likelihood (ML) analyses were conducted in RAxML via the Cipres Science Gateway Web Portal (Miller et al. 2010), using a GTR+I+G model and 1,000 bootstrap pseudoreplicates, with *Leucocytoon* assigned as the outgroup. We also performed an ML analysis of the same data set, but while constraining avian and mammalian *Plasmodium* parasites to be monophyletic. The resulting topologies were compared by performing approximately unbiased (AU), Kishino-Hasegawa (KH), and weighted Shimodaira-Hasegawa (wSH) tests in the programs PAUP*(Swofford 2003) and CONSEL (Shimodaira and Hasegawa 2001) using 1,000 bootstrap pseudoreplicates and resampling of estimated log-likelihood approximation (RELL) (Hasegawa and Kishino 1994; Kishino et al 1990).

Results and Discussion

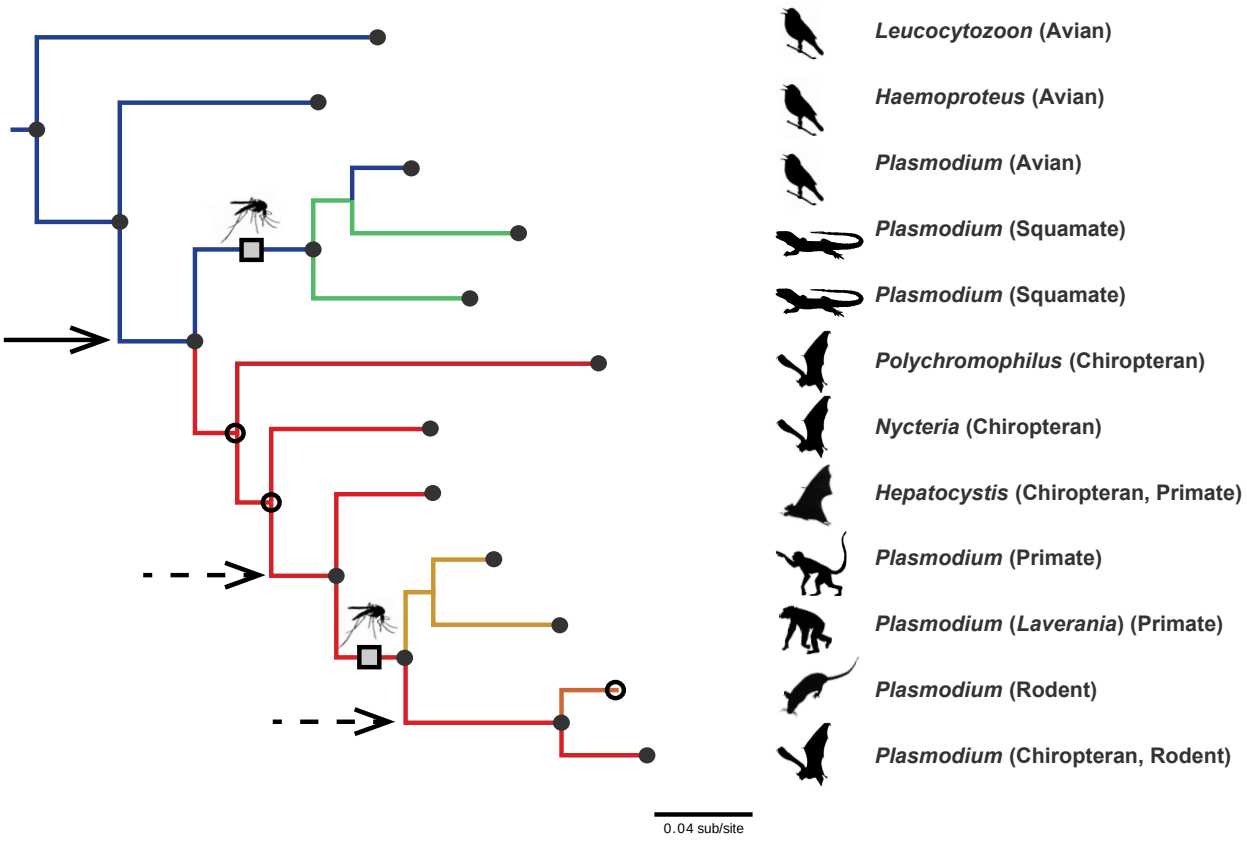


Figure 4.3. BEAST maximum clade credibility tree, based on four runs of 150 million generations with sampling every 5,000th tree (10% burn-in, 108,000 trees sampled, ESS > 1,000). Closed circle indicates > 99% posterior probability; open circle indicates > 95% posterior probability; dotted line indicates low support for clade. Colored triangles correspond to collapsed nodes of major haemosporidian parasite lineages from vertebrate hosts. Bold arrow indicates hypothesized transition from avian to mammalian hosts, and dotted arrows indicate transitions between chiropteran and primate or rodent hosts. Gray squares indicate identified lineages that exhibit erythrocytic schizogeny, which also happen to depend on mosquitoes as definitive hosts.

Our phylogenetic analysis of 170 haemosporidian parasite lineages from bats, birds, primates, rodents, and squamate reptiles supports the hypothesis that *Plasmodium* parasites of mammals evolved from parasites of chiropteran hosts (Figure 4.3). In contrast to most recent phylogenetic hypotheses (Borner et al. 2016; Martinsen et al. 2008b; Outlaw and Ricklefs 2011; Schaer et al. 2013; Schaer et al. 2015), our analyses strongly support the reciprocal monophyly of *Hepatocystis* and mammalian *Plasmodium* species (Figure 4.4). In further contrast to the

recent phylogenetic study of Borner et al. (2016), topology tests did not support the monophyly of avian and mammalian *Plasmodium* parasites (Table 4.2), rendering the genus paraphyletic.

Our analyses utilized genetic information from the broadest taxonomic sampling of Haemosporida currently available, and identified well-supported relationships that differ from analyses relying on large data sets of orthologous genes derived from a fewer number of haemosporidian taxa (e.g. Borner et al. 2016; Dávalos and Perkins, 2008). The effect of increasing genes versus taxa in phylogenetic reconstruction remains a controversial and important topic (Nabhan and Sarkar 2012), and a combination of both approaches will likely be required before arriving at our best estimate of the true evolutionary history of Haemosporida.

The topology of our tree, rooted with *Leucocytozoon*, provides modest support for a single invasion of haemosporidian parasites from sauropsids into mammals (BI = 0.95, ML = 53), with subsequent diversification among lineages of bat parasites and multiple invasions into other mammals. This emerging pattern of chiropteran parasites transitioning into non-chiropteran hosts, including primates and rodents, is observed both at deep and shallow levels in the phylogeny (Figure 4.4).

Specifically, we observe a transition of *Hepatocystis* parasites from Asian pteropodids (megabats) into Asian and African primates, and a similar transition of *Plasmodium* in African pteropodids into African rodents. The large genetic and geographic diversity of *Hepatocystis* lineages found in pteropodids and their paraphyletic relationship to *Hepatocystis* of primates suggest that these bats may be an important source of *Hepatocystis* parasites with the potential to shift into other mammal (e.g. primate) groups.

Table 4.2. Maximum Likelihood (ML) topology tests, comparing likelihood of unconstrained topology, and a constraint forcing the monophyly of all *Plasmodium* lineages.

Maximum Likelihood Topological Constraints	ln*L*	Δln*L*	KH test *p*-value	AU test *p*-value	SH test *p*-value	wSH test *p*-value
Unconstrained	-51,783.01	(best)	-	-	-	-
Plasmodium monophyletic	-52,148.49	365.49	< 0.0001	< 0.0001	< 0.0001	< 0.0001

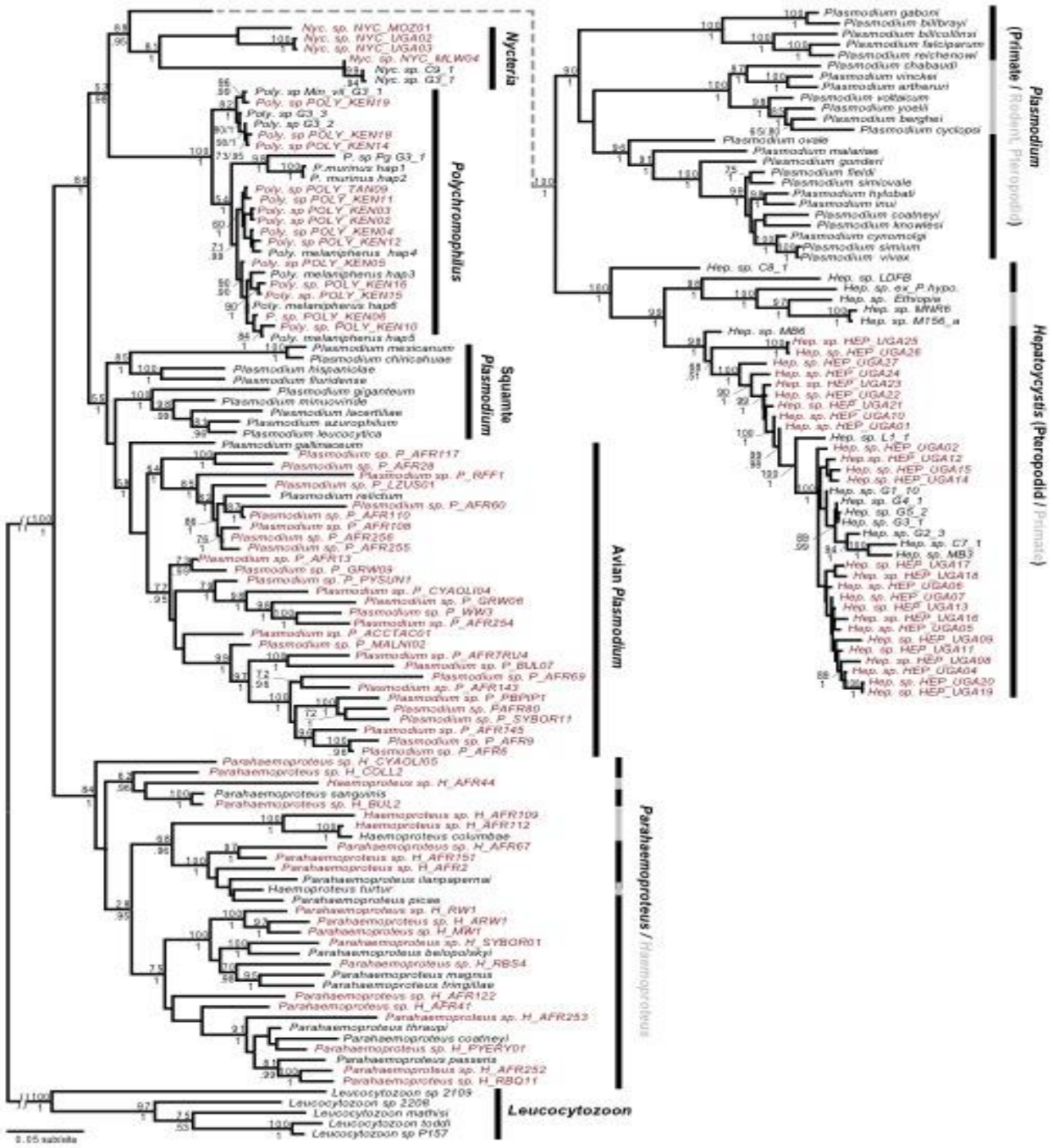


Figure 4.4. Maximum Likelihood (ML) phylogeny, rooted with *Leucocytozoon*. Support values indicated at nodes include ML support based on 1,000 bootstrap pseudoreplicates, and Bayesian inference (BI) posterior probabilities from maximum clade credibility tree summarized from four independent mcmc chains of 150 mil generations (above and below nodes, respectively). Terminal taxa labeled in red represent parasite lineages sequenced in this study.

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However, concentrated sampling of pteropodids in both East Africa (this study) and West Africa (Schaer et al. 2013) may be biasing the diversity we observe. Further sampling of Old World primates and Asian bats will likely improve the resolution of evolutionary host switches between bats and primates. On a larger phylogenetic scale, the sister relationship of *Polychromophilus* and *Nycteria* parasites (which exclusively infect bats) to the clade containing *Plasmodium* and *Hepatocystis* suggest that the ancestor of primate-infecting *Plasmodium* species was likely to be a chiropteran parasite.

We find strong support from both BI and ML analyses for a sister group relationship between *Hepatocystis* and *Plasmodium* (BI = 1, ML = 100), as well as the respective monophyly of each group (*Hepatocystis*: BI = 1, ML = 100; *Plasmodium*: BI = 1, ML = 90), which is contrary to previous phylogenetic hypotheses (Duval et al. 2012; Martinsen et al. 2008b; Schaer et al. 2013). This novel relationship may be attributable to our inclusion of recently discovered primate parasites in the subgenus *Laverania* (*P. billcollinsi*, *P. billbrayi*), which also contains the deadly human malaria parasite *P. falciparum* and other closely related parasites of the great apes. Our phylogeny suggests that the defining feature of true "malaria" parasites – erythrocytic schizogony, or the asexual reproduction in red blood cells of hosts – may have arisen twice in the haemosporidian Tree of Life (once in the saurian *Plasmodium* lineage, and once in the mammalian *Plasmodium* lineage), possibly in association with the dependence on mosquitoes as definitive hosts (Figure 4.3). Our placement of *Hepatocystis* as sister to mammalian *Plasmodium* eliminates the requisite assumption that erythrocytic schizogony was a trait lost in the *Hepatocystis* lineage (Borner et al. 2016; Martinsen et al. 2008b; Outlaw and Ricklefs 2011; Schaer et al. 2013), and instead suggests that this trait has convergently evolved. The correlation between erythrocytic schizogony and dependence on mosquitoes for transmission highlights an

intriguing area of study in the coevolution of haemosporidians and their definitive hosts (dipteran insects, which also serve as vectors). The evolutionary relationships between dipteran vectors and haemosporidian parasites have been poorly studied, but are presumed to be influenced by strong selective forces on both parasites and hosts (Valkiūnas 2005). Indeed, recent experiments demonstrated that the ingestion of avian *Haemoproteus* parasites (*H. balmorali*, *H. tartakovskyi*, and *H. lanii*) can be fatal to culicid mosquitoes, which are not the definitive hosts of these species (Valkiūnas et al. 2014). Such significant survival effects of, in this case, geographically widespread parasites on a similarly widespread vector, suggest that parasite life-history traits are tightly linked to their insect hosts, and underscore the need for additional studies in this area.

We found only 24% of the bat species sampled (21% of the individuals) to be infected by haemosporidian parasites, with those infected belonging to only 6 of the 21 genera sampled (Table 4.1). Notably, no other mammals (rodents or shrews) were found to be infected. Although the primers we used for molecular detection of parasites were able to amplify parasites from many diverse haemosporidian groups (e.g. *Haemoproteus, Hepatocystis, Leucocytozoon*, *Nycteria*, *Plasmodium*, *Polychromophilus*), it is possible that some parasites of rodents and shrews may have diverged sufficiently at the *cytb* locus such that we were unable to molecularly detect them. Microscopic analysis of a subset of rodent and shrew blood films (~100 and 50 randomly selected blood films of rodents and shrews, respectively), however, did not reveal any haemosporidian parasites. Microscopic analysis of PCR-negative blood films from bats also did not reveal haemosporidian parasites, whereas microscopy of PCR-positive samples confirmed the presence of morphologically-identifiable haemosporidians in all cases sampled (Figure 4.2). We emphasize that a thorough examination of all blood films – and not those of a subset only – collected for this study is needed to definitively conclude that no rodents or shrews were infected

by haemosporidian parasites. Parasite diversity in the bats we sampled included 50 unique lineages (unique lineages being defined, conservatively, as having a single nucleotide polymorphism at the *cytb* locus) (Appendix J). In stark contrast, a recent study using identical parasite detection methods and comparably broad taxonomic sampling found ~93% of bird species (80% of individuals) sampled in Malawi to be infected with 248 unique haemosporidian parasite lineages (Lutz et al. 2015). In general, broad taxonomic surveys of Afrotropical bats, both in our study and in others (Duval et al. 2012; Schaer et al. 2013; Schaer et al. 2015), indicate that only a small proportion of potential chiropteran host species harbor haemosporidian parasites, whereas the majority of potential avian host species in any given region are likely to be associated with one or more parasite lineages (Beadell et al. 2009; Belo et al. 2011; Ishtiaq et al. 2007; Lutz et al. 2015; Okanga et al. 2014; Ribeiro et al. 2005; Svensson-Coelho et al. 2013).

The difference in haemosporidian prevalence among species of birds and species of bats may be attributable to several factors, including ecological barriers to host–vector interactions that limit or accelerate opportunities for parasite transmission, and immunological responses in hosts that render them either viable or dead-end hosts for parasites. Coevolutionary hypotheses have been tested for several major lineages of haemosporidians, including mammalian and avian *Plasmodium* (Beadell et al. 2009; Jenkins et al. 2012; Silva et al. 2011, Silva et al. 2015). *Plasmodium* parasites of mammals exhibit high host specificity at the genus level (Garnham 1966), relative to avian parasites, which are strikingly labile with respect to host specificity (Valkiūnas 2005). Whereas comparative genomic studies of mammalian *Plasmodium* species reveal deep temporal connections between hosts and parasites (Silva et al. 2011, Silva et al. 2015), analyses of avian haemosporidians rarely find such relationships (Beadell et al. 2009; Hellgren et al. 2009), but rather, indicate tight links between ecology or host life history traits

and parasite associations (Fecchio et al. 2013; Jenkins et al. 2012; Lacorte et al. 2013). Whether ecology and life history influence the relationships between bats and their haemosporidian parasites remains to be seen. Host–parasite specificity of *Nycteria* (Schaer et al. 2015) and *Polychromophilus* (Duval et al. 2012) parasites does appear to be highly relative to avian host–parasite associations, but sampling of potential hosts for these two parasite genera has also been relatively low (as it has been with many other mammal groups). Few studies have examined feeding preferences of dipteran vectors of haemosporidians in wild bats (Witsenburg et al. 2015), and it is unknown how they might compare to those of avian hosts. Indeed, the dipteran vectors for most chiropteran parasites are unknown. The life history traits of chiropteran haemosporidians, however, suggest that host immunology represents an important factor in chiropteran parasite evolution. For example, *Hepatocystis* parasites exhibit prolonged developmental stages in the liver, with only brief periods of gametocyte circulation in the blood (Garnham 1966), which has been suggested to be an adaptation to the unique immunological environment and metabolic demands of chiropteran hosts (Schaer et al. 2013).

The suggestion that bats harbor exceptional prevalence and diversity of parasites and pathogens relative to other mammalian hosts (Luis et al. 2013; Schaer et al. 2013) may hold true as knowledge of parasite diversity among mammals increases. However, evidence from recent Afrotropical surveys suggests comparably high parasite diversity in other mammalian groups, such as primates (Duval et al. 2010; Krief et al. 2010; Liu et al. 2010; Ollomo et al. 2009; Prugnolle et al. 2010). Perhaps more interesting than the prevalence of chiropteran haemosporidians (21% in this study), which is low compared to avian parasite prevalence (~80% in the same region; Lutz et al. 2015), is the range of phylogenetic positions that they occupy in the haemosporidian Tree of Life. *Polychromophilus* and *Nycteria* parasites are the closest known

mammal-infecting relatives of avian haemosporidians, the chiropteran *Plasmodium* parasites *P. voltaicum* and *P. cyclopsi* are sister to the rodent *Plasmodium* species, and chiropteran *Hepatocystis* parasites are sister to the primate-infecting *Hepatocystis* species. As noted in previous studies of pathogen spillover from bats (Calisher et al. 2006; Dobson 2005), the exceptional nature of chiropteran pathogens lies not in their diversity, but rather, in their pronounced pathogenicity once they transition to novel hosts – a feature possibly linked to the evolution of flight in bats (O'Shea et al. 2014; Zhang et al. 2013). Such evolutionary forces acting on bats and their pathogens may very well be driving the apparent phylogenetic mobility of chiropteran haemosporidians.

Conclusion

The study of haemosporidian diversity and evolution has benefited from the steady efforts of biologists seeking to explore new host–parasite associations throughout the world. The importance of taxon sampling when assessing evolutionary relationships cannot be understated, and in this study, we present a novel hypothesis for haemosporidian parasites based on improved sampling of both saurian and mammalian hosts from the Afrotropics. In the future, sampling of haemosporidians from other additional geographic locations and host taxa (e.g. Asian mammals, New World bats, ungulates, etc) should continue to improve our understanding of the evolution of parasites in this extraordinary group. Our phylogenetic analyses produced strong support for relationships at major nodes within the haemosporidian tree, and did not support the monophyly of *Plasmodium* parasites from birds and mammals. The consistent and strongly supported placement of *Polychromophilus* and *Nycteria* parasites as sister to mammalian *Plasmodium* and

Hepatocystis suggest that malaria parasites in primates and rodents are derived from an ancestor infecting chiropteran hosts. The transition of parasites from chiropteran to non-chiropteran hosts appears to be a pattern throughout the evolutionary history of mammalian haemosporidian parasites, and may be linked to ecological or immunological factors that are unique to bats.

ACKNOWLEDGEMENTS

We would like to provide a special acknowledgement to our co-author, Dr. William "Bill" T. Stanley, who was instrumental in both the initiation and completion of this project. Dr. Stanley passed away unexpectedly, shortly after the completion of this study, while conducting field work in Ethiopia. His keen insights, passion for scientific research, and deep knowledge of African mammals made him a valuable and respected collaborator. We also thank the many staff members of the parks and museums with which we collaborated throughout the duration of this project, including Museums of Malawi (MM), the Malawi Department of National Parks and Wildlife, Gorongosa National Park (GNP), Makerere University Museum of Zoology (MUMZ), the Uganda Wildlife Authority (UWA), Karatina University (KU), Ol Jogi Wildlife Conservancy, National Museums of Kenya, and the Kenya Wildlife Service (KWS), the Tanzania Wildlife Research Institute (TAWIRI), Southern Highlands Conservation Programme (SHCP) and the University of Dar es Salaam (UDSM). Specifically, we thank Waswa Sadic, Cecelia Akoth, and Robert Kityo (MUMZ), Charles Akora (UWA), Samuel Kasiki (KWS), Beryl Makori and Dedan Ngatia (KU), Nyson Gawani and Jimmy Mussa (MM), Tongai Castigo, Alan Short, and Franziska Steinbruch (GNP), Philip Kihaule (UDSM) and Maiko Munissi (SHCP), and Josh Engel (FMNH) for their aid and dedicated efforts in the field. We also thank José Patané, Corrie Moreau, and Jason Weckstein for their helpful conversations regarding phylogenetic analyses, Kara Taylor, Heather Skeen, and Erica Zahnle for their assistance in the lab, and the anonymous reviewers who helped clarify and improve this manuscript. This project was supported by the Field Museum of Natural History's Emerging Pathogens Project, funded by the Davee Foundation and the Dr. Ralph and Mariam Faulk Medical Research Trust, and by

grants from the Athena Fund at the Cornell Lab of Ornithology and the Gregory C. Carr Foundation. Support from the NSF Malaria Research Coordination Network also aided in fostering and maintaining the collaborations required during this project. DNA sequencing was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum of Natural History. Lastly, we are grateful for the use of the CIPRES Science Gateway and the NSF XSEDE project, which helped make this project possible.

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CHAPTER 5

LASER CAPTURE MICRODISSECTION MICROSCOPY AND GENOME SEQUENCING OF THE AVIAN MALARIA PARASITE, *PLASMODIUM RELICTUM*[4]

Abstract

Acquiring genomic material from avian malaria parasites for genome sequencing has proven problematic due to the nucleation of avian erythrocytes, which produces a large ratio of host to parasite DNA (~1 million to 1 bp). We tested the ability of laser capture microdissection microscopy to isolate parasite cells from individual avian erythrocytes for four avian *Plasmodium* species, and subsequently applied whole genome amplification and Illumina sequencing methods to *Plasmodium relictum* (lineage pSGS1) to produce sequence reads of the *P. relictum* genome. We assembled ~335 kbp of parasite DNA from this species, but were unable to completely avoid contamination by host DNA and other sources. However, it is clear that laser capture microdissection holds promise for the isolation of genomic material from haemosporidian parasites in intracellular life stages. In particular, laser capture microdissection may prove useful for isolating individual parasite species from co-infected hosts. Although not explicitly tested in this study, laser capture microdissection may also have important applications for isolation of rare parasite lineages and museum specimens for which no fresh material exists.

[4] Lutz HL, Marra NJ, Grewe F, Carlson J, Palinauskas V, Valkiūnas G, Stanhope MJ. In Review. Laser capture microdissection microscopy and genome sequencing of the avian malaria parasite, Plasmodium relictum. Parasitol. Research.

Introduction

Haemosporidian parasites (Apicomplexa: Haemosporida), including malaria-causing parasites in the genus *Plasmodium*, are geographically ubiquitous and infect nearly all terrestrial vertebrates (Garnham 1966; Telford 2009; Valkiūnas 2005). They are obligate intracellular parasites that can infect many different host cells of various tissues throughout their life cycle. Wild vertebrate hosts, such as lizards, birds, and bats, are frequently infected with multiple species of haemosporidian parasites, and this can interfere with molecular analyses of individual parasites. Furthermore, the intracellular nature of haemosporidian parasites makes extracting their DNA without capturing the DNA of their hosts a significant challenge.

Laser capture microdissection microscopy (LCMM) holds great promise for the study of single haemosporidian cells, and may have important applications for the study of coinfections by two or more parasites, as well as isolation of parasite DNA for genomic analysis. Genome sequencing of haemosporidian parasites has and will continue to aid the characterization of gene content differences and variation between parasite lineages, thereby providing important insights into the molecular adaptations underlying specific traits such as virulence factors, life histories, and host immune evasion strategies. In addition to informing such comparative genomic studies, the sequencing of haemosporidian genomes – and genomes of avian malarial parasites in particular – will provide much needed sequence data for the development of new phylogenetic markers. These markers will in turn allow greater insight into the evolutionary history of this ancient and diverse group of apicomplexan parasites (Perkins 2014). In particular, the development of nuclear markers is expected to help resolve deeper phylogenetic relationships in

the malaria Tree of Life (Perkins 2014). The development of molecular barcodes (mainly 16S and *cytb*) and increased sampling of wild animals have revealed phenomenal diversity among haemosporidian parasites (Lutz et al. 2016; Prugnolle et al. 2010; Schaer et al. 2013). Genetic diversity within the genus *Plasmodium* is exceptionally high, especially among parasite species that infect birds (Clark et al. 2014; Harrigan et al. 2014; Ishtiaq et al. 2012; Lutz et al., 2015). Although molecular barcodes have aided in the discovery of a great number of parasite lineages, they have not provided enough data to resolve many of the evolutionary relationships between parasite species at either deep or shallow nodes in the phylogenetic tree. Furthermore, many host and geographic regions still remain to be explored for malarial parasite diversity. Thus, a combination of improved taxonomic sampling and addition of phylogenetically informative markers is expected to improve the resolution of deep evolutionary relationships.

Avian *Plasmodium* parasites are of particular interest to malaria systematists due to their widespread distributions among disparate hosts and geographic regions, as well as their purported relationship as sister to all mammalian haemosporidians (Lutz et al. 2016; Schaer et al. 2013). Birds and reptiles host the majority of known *Plasmodium* spp. diversity in the haemosporidian Tree of Life (Garnham 1966; Valkiūnas 2005). Unlike mammals, birds and reptiles have nucleated red blood cells, and the ratio of host to parasite genome size in the case of avian malaria is ~1.2 Gbp:25 Mbp (*Serinus canaria*: *Plasmodium*). In addition to the discrepancy in genome size, parasitemia tends to be low in naturally infected birds and reptiles, further skewing the ratio of host to parasite DNA. Lastly, coinfections by multiple haemosporidian species are common in these hosts (Valkiūnas 2005) and can complicate studies of parasite DNA extracted from whole blood. All of these factors have contributed to the paucity of genomic data from sauropsid haemosporidians, which has hindered most phylogenetic analyses to date. This

necessitates the development of novel methods for obtaining genomic sequence data to conduct comparative analyses, and this provides a good opportunity to test the ability of laser capture microdissection microscopy technology (LCMM) methods to isolate parasite DNA by extracting individual parasites directly from the nucleated cells of their hosts.

To this end, we have paired LCMM and whole genome amplification to obtain genomic DNA of sufficient quality from avian *Plasmodium* parasites for next generation sequencing methods. Using LCMM, we obtained DNA from thin blood smears representing three subgenera of avian *Plasmodium* species: *Plasmodium (Haemamoeba) relictum*, lineage pSGS1; *Plasmodium (Haemamoeba) cathemerium*, SEIAUR01; *Plasmodium (Giovannolaia) homocircumflexum*, pCOLL4; *Plasmodium (Novyella) ashfordi*, pGRW2. We successfully recovered a standard 478 bp Cytochrome b (*cytb*) barcoding fragment from all species following LCMM, and further sequenced genomic DNA of *P. relictum* using an Illumina MiSeq. Although we were unable to completely eliminate host contamination, our results demonstrate the utility of thin blood smears as a resource for the genomic study of malaria parasites and other haemosporidians, and support LCMM as a viable method for isolating individual malarial parasite cells for downstream sequencing. Previously, it was speculated that laser-isolated material would not be of sufficient quality for genomic sequencing or nuclear marker development. In this study, we demonstrate that laser-isolated material is indeed sufficient for such analyses. These results have important implications for the study of other intracellular parasites, and hold promise for dealing with the challenge of multi-parasite coinfections by allowing researchers to isolate, amplify, and sequence individual parasite cells.

Methods

Obtaining blood stage parasites

Plasmodium relictum (cytochrome *b* lineage pSGS1), *Plasmodium ashfordi* (lineage pGRW2), *Plasmodium homocircumflexum* (lineage pCOLL4), and *P. cathemerium* (lineage SEIAUR01) strains were used in this study. Collection methods of the blood stages of *P. cathemerium* (SEIAUR01) are detailed in Carlson et al. (2016). *P. relictum*, *P. ashfordi*, and *P. homocircumflexum* strains were originally isolated from a common crossbill *Loxia curvirostra*, common cuckoo *Cuculus canorus* and red-backed shrike *Lanius collurio*, respectively. Birds were sampled on the Curonian Spit in the Baltic Sea in 2010 and 2011. The original parasite isolates were multiplied by blood passages and cryopreserved in liquid nitrogen for long-term storage, as described by Palinauskas et al. (2015). In total, strain SGS1 underwent 4 passages in crossbills and domestic canaries (*Serinus canaria domestica*), pGRW2 underwent 2 passages in siskins (*Carduelis spinus*), and pCOLL4 underwent 6 passages in crossbills and siskins after the original isolation before this study. To obtain blood stages for laser microdissection, strains were further multiplied by infecting intact domestic canaries. The work was carried out in the Institute of Ecology, Nature Research Centre between February and March 2014. The birds were commercially purchased and tested (prior to experimental exposure) for the presence of possible natural malarial infection both by microscopic examination of blood films and PCR-based methods, as described by Palinauskas et al. (2015).

Two uninfected canaries were exposed to *P. relictum* (pSGS1) infection by inoculation of infected blood mixture into pectoral muscles, as described by Palinauskas et al. (2008). All birds

were kept indoors in a vector-free room under controlled conditions (55%–60% relative humidity, 20±1 ºC, the natural light–dark photoperiod), and they were fed standard diets for seed-eating bird species. Ten uninfected canaries were maintained in the same room, and used as negative controls to prove the absence of malaria transmission in the laboratory.

To follow the development of parasitemia, blood for microscopic and molecular examination was taken from all canaries every two or three days post inoculation (dpi) for approximately one month. A drop of blood was collected by puncturing the brachial vein to make two blood films for microscopic investigation, and about 30–50 µl of blood was saved in micro-tubes in non-lysis SET-buffer (0.05 M tris, 0.15 M NaCl, 0.5 M EDTA, pH 8.0) for molecular analysis (PCR and Sanger sequencing of *cytb* for verification of parasite identity), which was performed according to Palinauskas et al. (2015), and confirmed the presence of the lineage pSGS1 in the experimentally exposed canaries. Blood films were prepared, air-dried, fixed with absolute methanol, stained with Giemsa and examined microscopically, as described by Valkiūnas et al. (2008).

All control canaries remained uninfected. Two experimental birds developed parasitemia. Fourteen days post infection (dpi), parasitemia of *P. relictum* reached 12.5% in one infected canary, and thin blood films were made from this bird on ZEISS 0.17 PEN MembraneSlides objective slides for laser microdissection. The blood films were fixed with 70% ethanol, dried and stored in +4 °C until further processing.

Experimental procedures for this study were approved by the Ethical Commission of the Baltic Laboratory Animal Science Association (Lithuania) and Lithuanian State Food and Veterinary Office (Ref. no. 2012/01/04-0221), Lithuania. Experimental procedures conducted in the United States complied with IACUC permit 17601 UC Davis.

Laser capture microdissection microscopy and purification of parasite DNA

All *Plasmodium* cells were isolated from unstained ZEISS 0.17 PEN MembraneSlides, using a ZEISS PALM MicroBeam Laser Capture Microdissection (LCMM) system. Infected cells were identified by displacement of avian red blood cell nuclei – which is a characteristic feature of this parasite species' development (Valkiūnas 2005) – and the presence of parasite-derived hemozoin pigment granules (Figure 5.1). The region of interest (ROI), which included the parasite cell and excluded the host nucleus, was manually selected using PALM RoboSoftware (ZEISS). For laser microdissection of individual ROIs, the following settings were used: the cut energy = 45 – 50, LPC Delta = 20, focus = 7, Delta = -6, 1%, and cut iterations = 2 reps. ROIs were extracted and catapulted by a single laser pulse into adhesive cap 0.6ml tubes, suspended above the microscope stage.

For the study of *P. relictum* (pSGS1), we used LCMM to isolate varying numbers of cells. In independent rounds of LCMM, we isolated 1, 5, 10, 25, and 50 parasite cells, which we then subjected to a Chelex (Bio-Rad, Hercules, California) DNA extraction method, following the protocol described by Palinauskas et al. (2010): we inverted each tube of isolated cells and added 25 µl of freshly prepared Chelex suspension solution (0.2 g Chelex, 1000 µl H_2O, incubated at 56 ˚C for one hour) and 0.7 µl of Proteinase K to the lid of each tube. While remaining in this inverted position, tubes were incubated for one hour in a 56 ˚C water bath and gently vortexed every 20 minutes. After completing this incubation period, sample tubes were further incubated in water at 100 ˚C for 12 minutes to inactivate Proteinase K.

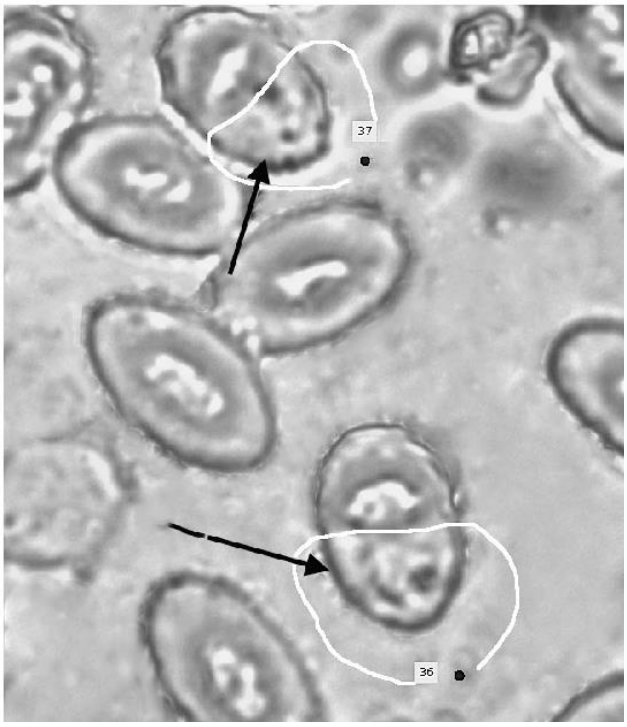


Figure 5.1. Microphotographs of avian erythrocytes (*Serinus canaria domestica*) infected with *Plasmodium relictum* (black arrows and ROI outline). Without the aid of a hematological stain (such as Giemsa), parasites were identified by the displacement of the host cell's nucleus and/or the presence of hemozoin pigment granules, which are caused by the digestion of the host cell's hemoglobin.

Following successful LCMM isolation and DNA extraction from *P. relictum* (pSGS1) samples, the same LCMM and extraction methods were applied to three additional *Plasmodium* species from different subgenera [provided by the experiments of Carlson et al. (2016)]. These included *P. (G.) homocircumflexum* (lineage pCOLL4), *P. (N.) ashfordi* (pGRW2), and *P. (H.) cathemerium* (pSEIAUR01). For each of these additional three species, 30 parasite cells were isolated via LCMM, and DNA from each parasite species was extracted using the same Chelex protocol described above.

Verification and whole genome amplification of Plasmodium isolates

To verify the identity of each LCMM-isolated parasite, we amplified 478 bp of *cytb* gene using a standard nested-PCR protocol (Waldenstrom et al. 2004), with both positive and negative PCR controls. PCR products were purified by enzymatic ExoSAP-IT (Affymetrix) and sequencing reactions were run with ABI Prism DYE Terminator Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase FS (Perkin-Elmer). Each reaction was then run on an ABI 3730 Automated DNA sequencer (Applied Biosystems, Foster City, California). Forward and reverse sequences were aligned and manually edited using Geneious v.6.1.6 (Biomatters Ltd., Auckland, New Zealand).

To produce sufficient DNA quantity for downstream preparation of a sequencing library, purified genomic DNA from the 50 cell-count extraction of *P. relictum* was amplified using a Qiagen REPLI-g Mini Kit, following the manufacturer's protocol. As with the original DNA extractions, whole genome amplified (WGA) product was also subjected to PCR and Sanger sequencing to verify the identity of the parasite. DNA was quantified following WGA using a Qubit® dsDNA Broad Range Assay Kit and Qubit® 2.0 Fluorometer (Invitrogen, ThermoFisher Scientific, Waltham, Massachusetts).

Sequencing and assembly of Plasmodium relictum genomic DNA

An Illumina TruSeq DNA Library Prep Kit (Illumina, San Diego, California) was used to prepare a 2 x 250 bp paired-end library using the WGA sample of *P.* relictum. The library was then sequenced on an Illumina MiSeq platform at Cornell university's Institute of BiotechnologyGenomics Core facility. Paired-end sequence reads of 250 bp were generated from sequencing libraries with a median insert of 750 bp. Adaptors were removed and raw reads were

quality trimmed using Trimmomatic v0.33 (Bolger et al. 2014) by removing 5' and 3' nucleotides with < 10 Phred score. The remaining reads needed to be greater than 35 bp and unpaired reads were removed. To assess the extent to which parasite sequences were obtained and host contamination eliminated, the remaining paired-end reads were separately mapped to an annotated *P. relictum* genome sequence provided by the Sanger Institute (GenBank BioProject PRJEB2579) and to the canary host genome sequence (NCBI SRA accession number ERS610854). Initial mapping of reads was done with the Bowtie2 mapper (Langmead and Salzberg 2012) using the default configuration. Since multiple chimeric reads consisting of parts of *P. relictum* or canary and sequences of other possible origin were unmapped by Bowtie2, we also used a less stringent blastn mapping method. For all reads used in blastn searches, default parameter settings were used to identify matches of each read to either the *P. relictum* genome, the Canary genome, or both genomes as combined subjects (indicating chimeric reads), respectively. Blastn results were used to filter all fastq paired reads by a custom perl script to produce datasets comprised of reads matching the *P. relictum* genome, the Canary genome, and all remaining reads without a match to either. The fastq sequences from each of these datasets were then used for individual *de novo* assemblies with SPAdes v.3.1.1 (Nurk et al. 2013). The resulting contig quality was measured with QUAST (Gurevich et al. 2013) (Table 5.1).

Table 5.1. QUAST measures of SPAdes *de novo* assembly of reads with hits to *P. relictum* genome

# contigs (> = 0 bp)	388
# contigs (> = 1000 bp)	65
# contigs (> = 5000 bp)	7
# contigs (> = 10,000 bp)	2
# contigs (> = 25,000 bp)	0
Total length (> = 0 bp)	335,639 bp
Total length (> = 1000 bp)	161,852 bp
Total length (> = 5000 bp)	53,338 bp
Total length (> = 10,000 bp)	22,089 bp
Total length (> = 25,000 bp)	0

Largest contig	11,536 bp
Total length	261,271 bp
GC (%)	19.91%
N50	1,511
N75	733
L50	40
L75	106
# Ns per 100 kbp	0

To examine the content of the *P. relictum de novo* assembly, we closely inspected the two largest contigs of the *P. relictum* assembly by blast searches against the reference genome and using the annotation functions implemented in Geneious v.8.1.8. To evaluate the origin of contigs assembled from reads that did not have any positive blastn match, contigs were blastx searched against the NCBI non-redundant database (max_target_seqs 5, word_size 5).

Results

LCMM isolation and PCR amplification of Plasmodium species

LCMM and Chelex extraction of all four *Plasmodium* species produced material that was sufficient for PCR amplification of 478 bp barcoding region of *cytb*, and Sanger sequencing confirmed the correct identity of each parasite extracted. This was true for all cell-counts (1, 5, 10, 25, and 50 cells) of *P. relictum*, as well as the 30 cell-count samples from the three other species (*P. ashfordi*, *P. cathemerium, P. homocircumflexum*). Qubit quantitation of DNA following WGA of the 50 cell-count *P. relictum* extraction indicated a final quantity of 1,274 ng of genomic DNA (18.2 ng/µl * 70 µl of sample) for preparation of the sequencing library (Qubit readings prior to WGA indicated a DNA concentration of <0.01 ng/µl).

Genome sequencing and assembly of P. relictum Illumina reads

0 Host (canary) contamination found in reads from Illumina sequencing of the WGA 50 cell-count *P. relictum* DNA was non-negligible, comprising ~22.57% of all reads, while only 0.07%

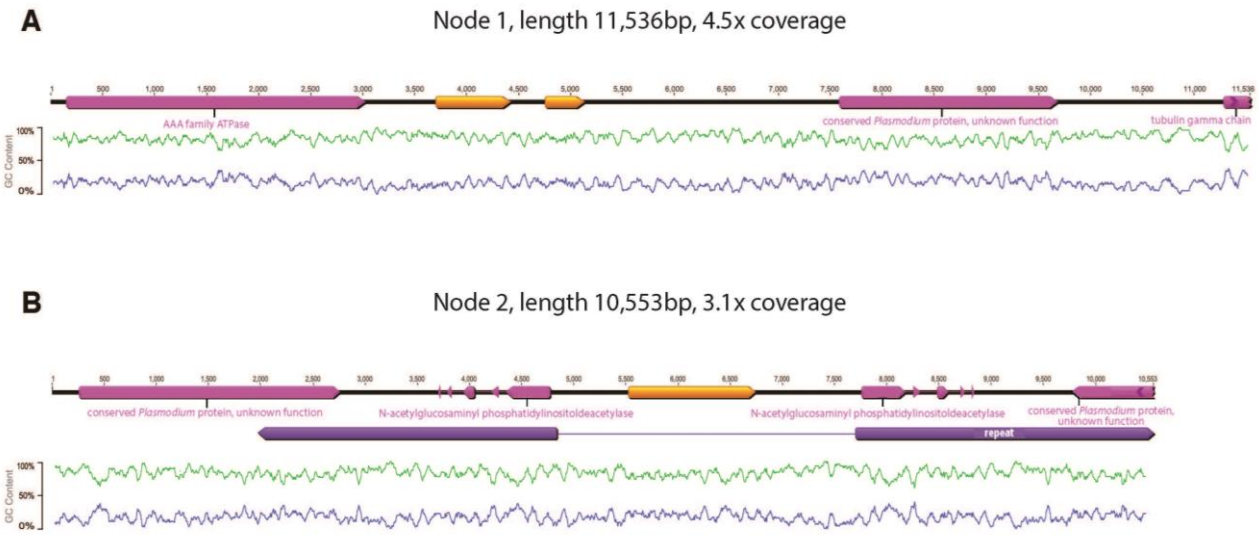
Table 5.2. Summary of Illumina MiSeq reads derived from 50-cell count LCMM isolate. Reads are characterized according to results from Bowtie mapping and BLASTN searches.

Total Raw PE-reads	9,064,941 reads		
Quality-trimmed PE-reads	8,496,876 reads		
	P. relictum (% of trimmed reads)	Canary (% of trimmed reads)	Other contaminant (% of trimmed reads)
Bowtie mapping	1,957 (0.02)	993,847 (11.7)	NA
BLASTN	6,295 (0.07)	1,917,989 (22.57)	6,574,823 (77.38)

of reads matched *P. relictum* (based on blastn searches against the canary and *P. relictum* genomes, respectively) (Table 2). Thus, avian host contamination was not completely eliminated by the use of LCMM. Other contaminants were determined to be primarily of bovine, bacterial, or fungal origin, and comprised the vast majority of sequencing reads (77.38%). All reads that were determined to be of a non-*Plasmodium* origin were excluded from the *P. relictum de novo* assembly.

Despite the low percentage of Illumina reads with blastn matches to the *P. relictum* genome, we were able to assemble these reads to produce 388 contigs (total length 335,639 bp). Contigs > 1000 bp comprised ~19% of all assembled contigs (Table 5.1). Blastx searches of all contigs from this *de novo* assembly against the NCBI nr database revealed that ~60% had significant hits to known *Plasmodium* sequences. The remaining 40% of contigs may represent

genes specific to *P. relictum* that are not present in the nr database. Examination of the two largest contigs (> 10,000 bp) revealed 1) an 11,536 bp protein-coding sequence containing a *Plasmodium* ATP synthase gene as well as a conserved *Plasmodium* protein-coding sequence of unknown function, and 2) what appears to be an inverted head-to-tail repeat of sequence coding for N-acetylglucosaminyl phosphatidylinositoldeacetylase with introns and a *Plasmodium* protein of unknown function (Figure 5.2).



Discussion

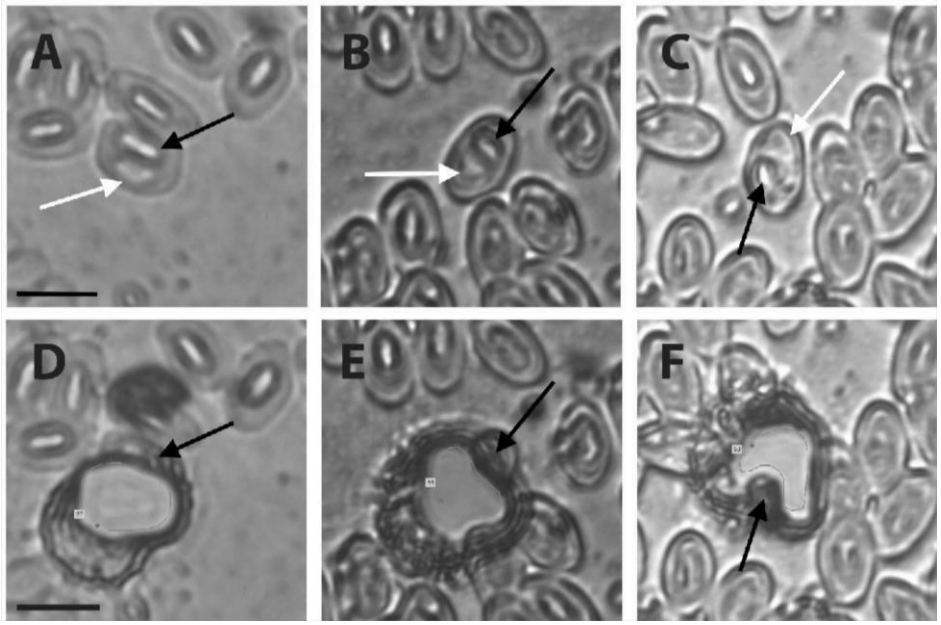
Figure 5.2. A–B. Gene content of two largest contigs from *P. relictum do novo* assembly. Open reading frames with blast hits to *P. relictum* (ERS610854) are indicated in magenta, other potential open reading frames are shown in orange. Characteristic high AT versus GC content is plotted below the annotation by green and blue lines respectively. (A) 11,536 bp contig (Node 1) containing ATP synthase coding gene and (B) 10,553 bp contig (Node 2) containing the N-acetylglucosaminyl phosphatidylinositol-deacetylase gene with introns. The head-to-tale inverted repeat of Node 2 could be an artifact of chimerization during whole genome amplification.

The results presented in this study suggest that LCMM may serve as a useful tool for isolating individual parasite cells for downstream molecular analyses, albeit without eliminating contamination from adjacent host nuclei. We were able to amplify 478 bp of *cytb* from a single

cell of *P. relictum*, as well as from 5, 10, 25, and 50 cell-count extractions for this species, following Chelex extraction and PCR amplification of LCMM-derived material. PCR amplification of 478 bp of *cytb* was also successful for 30-cell DNA extractions from three other species, including *P. ashfordi*, *P. cathemerium*, and *P. homocircumflexum* (for which single cell DNA extraction and amplification was not attempted). Although our method was not tested on co-infected hosts, the fact that PCR amplification from a single isolated cell of *P. relictum* was successful suggests that LCMM could be applied in studies where host individuals are co-infected by multiple haemosporidian parasites to isolate and differentiate between two parasite species. Illumina sequencing results of WGA *P. relictum* (50 cell-count LCMM isolate) were

Figure 5.3. A-D. Microphotographs of avian erythrocytes (*Serinus canaria domestica*) infected with *Plasmodium relictum*: (A-C) infected cell before laser capture microdissection; (D-F) the film after laser capture microdissection. Black arrows – avian erythrocyte nuclei, white arrows – *P. relictum* blood stages. Scale bar = 10 μm.

assembled successfully and produced 74 contigs > 1000 bp (9 of which were > 5000 bp, and 2 of which were > 10,000 bp). Inspection of these contigs revealed many potentially useful targets for the design of phylogenetic primers, although we did not explicitly produce primers or test the phylogenetic informativeness of these loci. One of the major goals of this experiment was to eliminate host contamination, and in this effort we were unsuccessful. It is likely that while targeting the intracellular *Plasmodium* parasites, portions of the host nucleus were also captured (see Figure 5.3). In addition to host contamination, a large portion of sequencing reads were also attributable to other contaminants, primarily of bovine and bacterial origin. It is unclear how these additional contaminants were introduced, but some experiments in this study were carried out in a multi-user facility in which such samples could possibly have been introduced (i.e. a microbiology laboratory that frequently contains bovine and bacterial samples). Our results suggest that more caution should be taken when working with LCMM-derived samples to avoid



such contamination, and that execution of LCMM (and downstream methods, such as whole genome amplification) should perhaps be carried out in a "clean" or "ancient DNA" laboratory setting. An additional anomaly in our sequencing reads was the existence of apparent chimeric sequences, including reads containing both parasite and host sequence (based on blastn searches), as well as abnormal parasite sequences that were assembled to form head-to-tail inverted repeats (see Figure 5.2). Again, it is unclear how such sequences were produced, but these results may be attributable to the process of whole genome amplification.

In previous attempts to isolate *P. relictum* using laser microdissection methods of single parasite cells (Palinauskas et al. 2010), the standard barcoding primers HAMENF/HAEMNR and HAEMF/HAEMR2 failed to amplify a long fragment of *cytb* (478 bp). However, a shorter fragment of *cytb* (224 bp) was successfully amplified in the experiments of that study. These results were attributed to the possible fragmentation of DNA during the laser isolation stage of the experiment. In this study, we were able to successfully amplify the full 478 bp barcoding fragment of *cytb* (regardless of the number of cells from which the template was derived), suggesting that at least mitochondrial genomic integrity is maintained during the LCMM

process. This may be expected, as the laser does not directly target the parasite cell, but rather, cuts around the periphery of the parasite (Figure 5.3). This also provides compelling evidence for the utility of LCMM as a method for isolating cells, in that clear progress has been made from the previous to the current experiments (Palinauskas et al. 2010).

In this study, we relied on specialized glass slides, but it is also possible to apply LCMM techniques to the standard glass slides that are commonly used by biologists and medical technicians for blood smear analysis (PALM Robo Software Manual, 2008). Thicker slides require a different application of laser power and focus, and if one is relying on ZEISS PALM Robo technology, regions of interest (e.g., parasite cells) must be targeted with individual laser pulses rather than by selection of a path around the target region. This may limit the current utility of LCMM methods when applied to blood smears prepared on standard glass slides, but this remains to be tested. Regardless, it is reasonable to expect that this potential limitation will be overcome with the refinement of laser microdissection technology.

With respect to the application of LCMM to museum specimens, another potential limitation of applying LCMM to museum-curated blood films is the effect of Giemsa (or other) stains on DNA quality. Evidence from a former LCMM-based study suggested that such stains may interfere with DNA amplification (Palinauskas et al. 2010). However, the ability to obtain DNA from Giemsa-stained slides of sufficient quality for molecular analysis has also been demonstrated (Ribeiro et al. 2005). Furthermore, protocols exist for removing histological stains (Bancroft 1967; Stevens and Francis 1996), making it unlikely for stains to be a truly limiting factor in molecular analyses of malaria parasites from blood smears.

Overall, we show that laser capture microdissection microscopy is a useful resource for isolating individual *Plasmodium* parasite cells from avian erythrocytes. This isolation method,

paired with whole genome amplification from multiple isolated cells, is sufficient for recovering large numbers of loci from the parasite's nuclear genome, which may be useful for molecular systematic studies of malaria parasites. Limitations of this method, with respect to histological stains and slide thickness, remain to be tested. Host (and other) contamination may also still present a challenge, as clearing the host nucleus of avian red blood cells when isolating parasites is very difficult and is limited by laser technology. Lastly, the formation of chimeric sequences, which may have occurred during the whole genome amplification process, is a non-trivial problem that could reduce the quality of assembled reads if not bioinformatically filtered out prior to assembly. Despite these challenges, it is clear that the ability of laser capture methods to produce a number of loci from a small number of cells holds promise for the development of new phylogenetic markers, comparative genomics, and perhaps even the investigation of museum specimens of rare or novel blood parasites. This method may also prove valuable to the investigation of natural and experimental systems in which multiple species of malaria parasites co-infect a single host.

ACKNOWLEDGEMENTS

This project was completed with support from the Athena Fund and the Cornell Lab of Ornithology, and from the Field Museum of Natural History's Emerging Pathogens Project, funded by the Davee Foundation and the Dr. Ralph and Mariam Faulk Medical Research Trust. Funding was also provided by the European Social Fund under the global grant measure (VPI-3.1.-ŠMM-07-K-01-047) to the Lithuania Research Council. We would like to thank Johanna Dela Cruz, Paulina Pavinski Bitar, and Peter Schweizer for their assistance with technical aspects of LCMM, DNA extraction, and genome sequencing, respectively. We also thank the Sanger Institute for their provision of unpublished sequence data from *Plasmodium relictum*.

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CHAPTER 6

POSITIVE DARWINIAN SELECTION IN THE VARIABLE SURFACE ANTIGENS MEROZOITE SURFACE PROTEIN (*MSP-1*) AND ERYTHROCYTE MEMBRANE-ASSOCIATED ANTIGEN (*EMA*) OF THE AVIAN MALARIA PARASITE, *PLASMODIUM RELICTUM*

Introduction

The theory of evolution by natural selection relies on several important tenets, including 1) the production of more individuals in a population than can survive in a given environment, 2) the existence of heritable variation among those individuals, and 3) the ability of individuals with particular heritable phenotypic variations to differentially survive in a given environment (Darwin 1872; Wallace 1870). When individuals in a population are engendered with such advantageous mutations, they are more likely to survive and pass the mutation on to their offspring. Thus, an advantageous mutation is expected to spread throughout the population and become "fixed", reaching an allele frequency of 1 (Kimura 1962). In such cases, the mutation is considered to be under positive Darwinian selection. Tests for positive Darwinian selection (PS) provide a means for identifying advantageous mutations that underlie phenotypic responses in biological species, and can provide important insights into the selective pressures that have shaped their evolution.

The rise of whole genome sequencing and the broadening of taxonomic sampling in many groups have vastly increased the amount of genomic data that can be analyzed to identify instances of PS. Such is the case for malarial parasites in the genus *Plasmodium* (Apicomplexa: Haemosporida). Comparative genomics of malarial parasites in general have provided a powerful method for understanding patterns of evolution and adaptation in this genus (Cai et al. 2012; Carlton et al. 2002; Carlton et al. 2008; Gardner et al. 2002; Silva et al. 2011). To date, most studies have focused solely on parasites infecting mammals (rodents and primates), and understandably so, given the vast toll malarial parasites continue to take on human populations (World Health Organization World Malaria Report 2015). However, it is important to recognize that parasite diversity in other host groups, such as birds and reptiles, is much higher than in mammals (Garnham 1966; Valkiūnas 2005). Parasites affecting these taxonomic groups are therefore likely to provide significant insights into the processes of host invasion, host-switching, and virulence factors.

Due to the extreme difficulty associated with obtaining genomic DNA from avian or reptilian *Plasmodium* species, neither has been included in most comparative genomic studies. Indeed, until recently, genomic sequences from these parasites were not even available, with the exception of galliform parasite *Plasmodium gallinaceum*, for which a 3x draft genome was sequenced in 2007 (Frech and Chen 2013), but was found to be highly erroneous due to assembly errors; this genome is currently being resequenced (S. Bensch, personal communication). Despite these challenges, efforts by the Wellcome Trust Sanger Institute to isolate and sequence the avian malarial parasite *Plasmodium relictum* have recently been successful. Unlike the majority of known *Plasmodium* parasites, *P. relictum* maintains a nearly global distribution, and infects a broad range of avian hosts from disparate orders (Valkiūnas

2005). Thus, this parasite is of particular interest for the study of adaptation and natural selection in haemosporidian parasites, as it is likely to experience unusual and increased selection pressures by facing such a diversity of immune responses in its varied hosts.

Previous comparative genomic analyses of mammalian *Plasmodium* species have identified several major gene families implicated in pathogenicity and chronic persistence of malarial parasites. These genes encode proteins that are typically associated with the host cell membrane (and thus, directly interact with host cells), or are responsible for epigenetic regulation of transcription of surface protein-coding genes. Variable surface antigens (VSA) of *Plasmodium* parasites, for example, have been extensively studied in mammalian models due to their significant role in host immune response evasion and host cell invasion. In mammalian parasites, these antigens are encoded by a number of large gene families that are variably expressed throughout the course of a single infection. This variation in surface antigen expression allows the parasites to evade host immune cells, thereby allowing the persistence of parasites in a chronically infective state. In rodents and primates, the *pir* gene superfamily includes homologous genes of the *bir* (rodent *P. berghei*), *cir* (primate *P. cynomolgi*), *kir* (primate *P. knowlesi*), *yir* (rodent *P. yoelii*), and *vir* (primate *P. vivax*) families. In the deadliest human malarial parasite, *P. falciparum*, and the closely related chimpanzee parasite, *P. knowlesi*, VSA genes include *rif*/*stevor* gene family (~190 genes) and *surfin* gene family (10 genes). A number of other host cell invasion-linked proteins are also encoded by VSA genes, are putatively involved with the regulation of VSA genes, or are directly involved with host cell invasion. These include genes encoding apical membrane antigen (*ama*), apical merozoite proteins (*amp*), circumsporozoite surface proteins (*csp*), erythrocyte-binding-like proteins (*ebl*), merozoite surface protein (*msp*), and histone-lysine N-methyltransferases (*set*).

In this study, we utilized genomic sequence of the widespread avian *Plasmodium* parasite, *P. relictum* (lineage pSGS1) to perform the first comparative genomic analysis of malarial parasites that includes a passerine *Plasmodium* species. We sought to identify regions of the *P. relictum* genome that are under PS to shed light on interspecific variation in selection pressures faced by a generalist avian parasite as opposed to those that specialize in rodents or primates. Because phenotypes on which selection can act may be controlled both by changes in protein sequences or changes in the transcription of proteins, we focused our analyses on both VSA genes and genes that are linked to epigenetic regulation of parasite virulence factors. Such genes were expected *a priori* to contain sites under PS in *P. relictum* due to their intimate role in host cell invasion and immune response evasion, and given that divergence between avian and mammalian host biology is great (relative to within-mammalian divergences).

Methods

Selection of Plasmodium genes and identification of positive selection in putative orthologs

Plasmodium relictum (lineage pSGS1) coding sequences (CDS) were obtained from the Wellcome Trust Sanger Institute (WTSI) and Illumina MiSeq reads derived from parasite cells isolated by laser capture microdissection microscopy (LCMM) (Lutz et al. in review). To identify genes of interest (e.g. VSA genes), sequences were subjected to blastx searches against the NCBI nr/nt data and assigned gene ontology (GO) terms using the program BLAST2GO (Conesa et al. 2005; Götz et al. 2008). Genes that were assigned GO terms of interest (transcription or cell-membrane associated gene ontology terms) were extracted from WTSI

and/or LCMM *P. relictum* assemblies, and orthologous genes from additional *Plasmodium* species were identified via blastx searches to the OrthoMCL database, or by reciprocal best blast searches against a database comprised of eight *Plasmodium* genomes available via PlasmoDB (www.plasmodb.org, release 31 March 2016); these included *P. berghei* ANKA (rodent), *P. chabaudi* chabaudi (rodent), *P. cynomolgi* Strain B (primate), *P. knowlesi* Strain H (primate). In some cases, orthologs from additional *Plasmodium* species that are not available through PlasmoDB were obtained from GenBank.

Positive selection is defined as the case in which a non-synonymous nucleotide substitution becomes fixed in a species due to a functional advantage that the mutation engenders in that species. To identify cases of positive selection in the avian malarial parasite, *P. relictum*, we examined individual alignments of putatively orthologous genes from all available *Plasmodium* species. For each gene of interest, complete CDS sequences were aligned using the RevTrans 1.4 Server (Wernersson and Pedersen 2003). Gene alignments were visually inspected for quality and correct orientation of open reading frames. Following inspection, each alignment was subjected to a branch-site test of positive selection using the codeml package within PAML v4.8 (Yang 2007) with default parameters. A starting tree was provided with a topology based on the recent phylogenetic analysis of Lutz et al. 2016, and branch lengths were estimated during PAML analysis. We compared two models: H0, which assumed a neutral rate of evolution ($dN/dS = 1$), and H1, which allowed for variable rates of evolution ($dN/dS < 0$ or > 1) across branches and sites. Regions of sequence alignments that contained gaps were removed from codeml analyses using the "cleandata" option.

Examination of sites under positive selection and effects on protein structure and function

Following tests for positive selection, amino acid sequences were compared to assess homology and divergence near sites under selection. In some cases, we also examined amino acid sequence variation at functionally important sites that are considered to be highly conserved, regardless of selection test results. Protein structure of amino acid sequences were predicted using the Phyre2 web portal for protein modeling, prediction, and analysis (Kelley et al. 2015), and sequence conservation and mutational effects for positions of interest within each sequence were determined using the program SuSPect (Yates et al. 2014). We also assessed the location of putative active sites by identifying large pocket regions using the program fpocket v2.0 (Le Guilloux et al. 2009), and examined the extent to which residues were important for conservation of protein structure using the Phyre2 investigator program (beta version), which relies on an information-theoretic approach based on Jensen-Shannon divergence (Capra and Singh 2007). To explore sites under selection for which Phyre2 failed to predict protein structure, we used the program RaptorX (Wang et al. 2011) to predict the secondary structure of genes.

Results

Gene selection and test for positive selection

Blast2GO analyses identified > 2,000 genes with transcription or cell-membrane associated gene ontology terms, from which we selected a subset of genes (n = 18) implicated in the processes of host cell invasion or immune response evasion (Table 6.1). Of the 18 genes selected, five *Set*-domain (*Set-1*, *Set-3*, *Set-4*, *Set-7*, *Setvs*) and two *Sir*-domain (*Sir2a*, *Sir2b*)

containing genes were included, both of which serve an important role in the epigenetic regulation of virulence genes during invasion and occupation of the host cell. The VSA genes we selected included the apical membrane antigen (*ama-1*), apical merozoite protein (*amp*), circumsporozoite protein (*csp*), circumsporozoite-related antigen (*exp-1*), erythrocyte membrane-associated antigen (*ema*), merozoite adhesive erythrocytic binding protein (*maebl*), merozoite surface proteins (*msp-1, msp-8, msp-10*), parasite-infected erythrocyte surface protein (*pieps15*), and rhoptry-associated protein (*Rap1*). In addition to genes with GO terms of interest, two housekeeping genes involved in chromatin remodeling (*Snf2*) and vacuolar catalytic ATP synthase, subunit h (*vapH*) were selected as a methodological control, as these genes are not expected to be under significant selection (Table 6.1).

Tests for selection identified 6 out of these 20 genes as containing amino acid sites that are significantly under positive Darwinian selection ($p > 0.05$) (Table 6.2). These included *ama-1*, *ema*, *exp-1*, *maebl*, *msp-1*, and *setvs*. Upon further inspection of dN/dS (ω) values for each gene, four (*ama-1*, *exp-1*, *maebl*, *setvs*) were deemed to be spurious results due to extremely elevated ω values; ω values > 10 are considered to be indicative of spurious results that could be due either to high variability of sites across the gene and between taxa (i.e., low rates of synonymous substitution), or due to sequencing errors or poor sequence alignment (Schneider et al. 2009). Visual inspection of sequence alignments for these genes indicated that the spurious results were likely caused by low dS values. The remaining two genes with significant positive selection results (*msp-1*, *ema*) had reasonable ω values ($\omega < 10$), and were subjected to further inspection.

Table 6.1. Genes selected for tests of positive selection

Gene	Pber*	Pcha	Pcyn	Pfal	Pkno	Prei	Prel	Pvin	Pviv	Pyoe
Transcription/regulation of virulence genes										
Set-1	X	X	X	X	X	X	X	X	X	X
Set-3	X	X	X	X	X	X	X	X	X	X
Set-4	X	X	X	X	X	X	X		X	X
Set-7	X	X	X	X	X	X	X	X	X	X
Setvs			X	X	X	X	X		X	
Sir2a	X	X		X	X	X	X	X		X
Sir2b	X	X		X	X	X	X	X		X
Variable surface antigens										
ama-1	X	X	X	X	X	X	X		X	X
amp	X	X	X	X	X	X	X		X	X
csp	X			X	X	X	X		X	X
ema	X	X	X	X	X	X	X		X	X
exp-1	X	X		X	X	X	X		X	X
maebl	X	X	X	X	X	X	X		X	X
msp-1	X	X		X		X	X			X
msp-10	X	X		X	X	X	X			X
msp-8	X	X		X	X	X	X			X
pieps	X	X		X		X	X			X
rap	X	X	X	X	X	X	X		X	X
Snf2	X	X	X	X	X	X	X		X	X
vapH	X	X		X	X	X	X		X	X

*Pber = *P. berghe*i, Pcha = *P. chabaudi*, Pcyn = *P. cynomolg*i, Pfal = *P. falciparum*, Pkno = *P. knowlesi*, Prei = *P. reichenowi*, Prel = *P. relictum*, Pvin = *P. vinckei*, Pviv = *P. vivax*, Pyoe = *P. yoelii*

Table 6.2. Likelihood scores, p-values, and dN/dS (ω) for branch-site tests of selection

Gene	OrthoMCL ID	-lnL H0	-lnL H1	p-value	dN/dS (ω)
*ama-1**	OG5_147452	8529.126	1525.495	0.007	20.5
amp	OG5_173160	3889.53	3888.856	0.246	NA
csp	OG5_159725	3226.485	3226.485	1	NA
ema	OG5_146660	21165.629	21163.452	0.037	9.55
*exp-1**	OG5_167197	1774.461	1770.321	0.004	415.84
*maebl**	OG5_126854	17834.91	17829.594	0.001	998.95
*msp-1**	OG5_132161	17465.182	17461.621	0.008	2.6
msp-10	OG5_166171	3611.564	3611.564	1	NA
msp-8	OG5_166908	5519.499	5519.499	1	NA
pieps	OG5_146522	5078.654	5078.654	1	NA
rap	OG5_147397	4978.832	4977.036	0.058	NA
Set-1	OG5_127895	48598.901	48595.462	0.009	62.62
Set-3	OG5_153109	16293.914	16293.914	1	NA
Set-4	OG5_166871	4521.15	4521.15	1	NA
Set-7	OG5_140372	8799.011	8799.011	1	NA
*Setvs**	OG5_128830	19502.815	19500.643	0.037	55.62
Sir2a	OG5_127825	2725.008	2725.292	0.451	NA
Sir2b	OG5_130121	11263.872	11263.752	0.624	NA
Snf2	OG5_141761	10307.04	10307.04	1	NA
vapH	OG5_127797	3895.275	3895.275	1	NA

Analysis of msp-1 and ema genes

Branch-site tests of selection for *msp-1* and *ema* genes indicated that for each gene, there was significant selection occurring at multiple amino acid residues (*msp-1*: $p = 0.008$, $\omega = 2.6$; *ema*: $p = 0.037$, $\omega = 9.55$). The sequence alignment for *msp-1* included genes from a total of 17 taxa (*P. berghei*, *P. chabaudi*, *P. coatneyi*, *P. cynomolgi*, *P. falciparum*, *P. fragile*, *P. gallinaceum*, *P. hylobati*, *P. inui*, *P. knowlesi*, *P. malariae*, *P. ovale*, *P. reichenowi*, *P. relictum*, *P. simium*, *P. vivax*, *P. yoelii*). The average sequence divergence for this gene and these taxa, excluding regions in the alignment that included gaps, was 64.27% identity (sequence similarity), with the maximum divergence exhibited between the primate parasite *P. hylobati* and the avian parasite *P. relictum* (51.41% identity), and the minimum divergence exhibited between

the rodent parasites *P. berghei* and *P. yoelii* (93.22% identity) (Appendix L). The sequence alignment for *ema* included genes from 9 taxa (*P. berghei*, *P. chabaudi*, *P. cynomolgi*, *P. falciparum*, *P. knowlesi*, *P. reichenowi*, *P. relictum*, *P. vivax*, *P. yoelii*). The average sequence divergence for these sequences (excluding gaps) was 74.80% identity, with the maximum divergence exhibited between the avian parasite *P. relictum* and primate parasite *P. vivax* (65.54% identity), and the minimum divergence exhibited by the primate parasites *P. falciparum* and *P. reichenowi* (97.29% identity) (Appendix L).

Results from branch-site tests for *msp-1* identified five residues as being under significant positive selection (Figure 6.1 A). The first two of these were located near the amine-terminus of the protein at sites within an amino acid sequence coding for a transmembrane peptide (residues 125 and 340). Three additional sites were identified nearer the carboxyl-terminus of the protein, in regions exhibiting α-helix secondary structure, but for which the final product could not be predicted with sufficient confidence via Phyre2 (residues 1080, 1264, and 1328). Analysis of *msp-1* secondary structure by RaptorX revealed these three sites to be located on α-helix structures within the un-modeled section of the protein (Figure 6.2 A), and overall secondary structure for the complete protein was composed of 81% α-helix, 2% β-sheet, 17% loop structures, with 18% of the protein structure qualifying as disordered (i.e., unpredictable secondary structure). The predicted protein structure of the transmembrane protein domain that

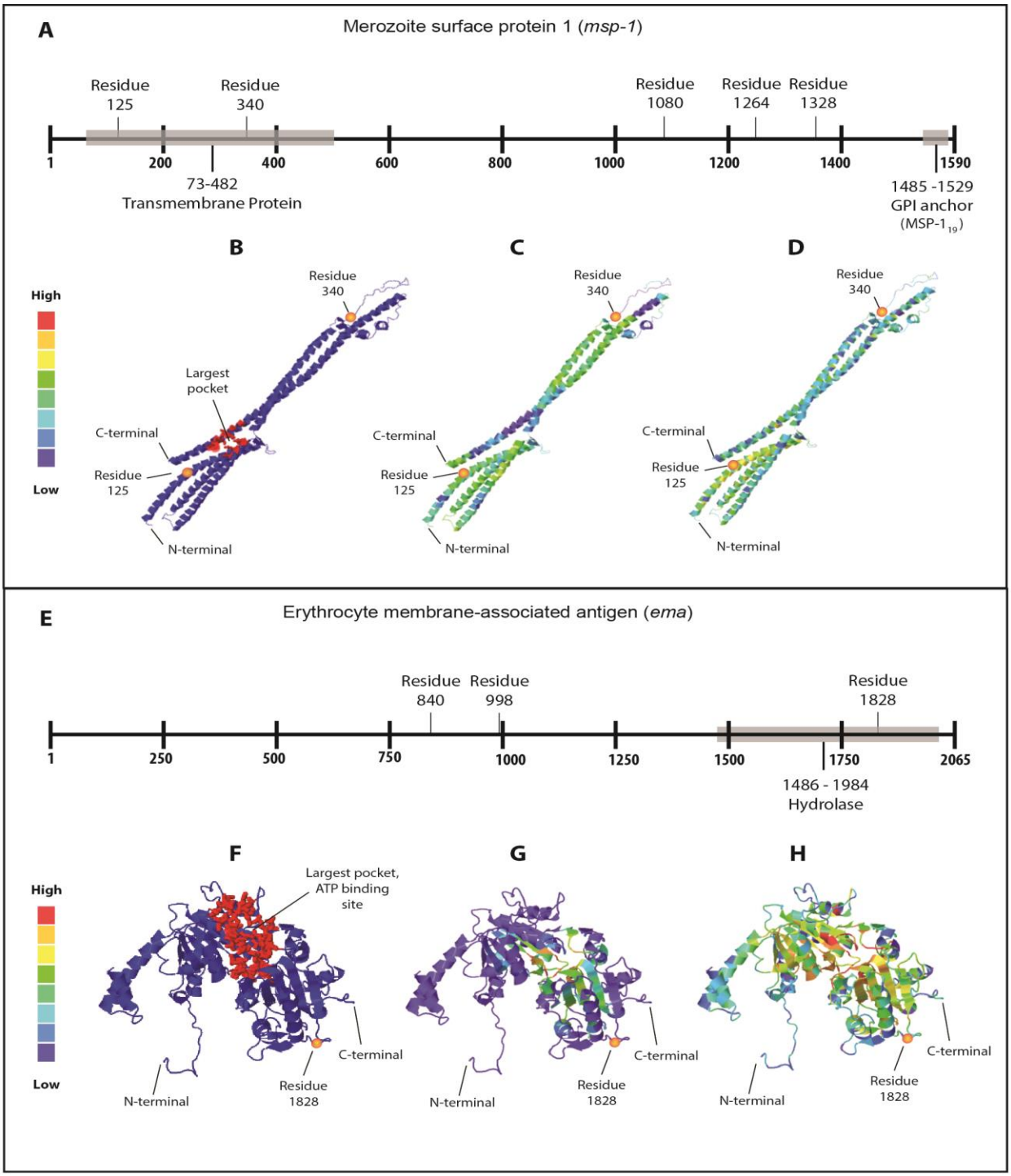

Figure 6.1. Phyre2 predicted protein domains and structures for *msp-1* (A–D) and *ema* (E–H). Residues under positive selection (PS) are annotated on the linear amino acid sequence schematic for each gene, and protein structures for domains containing residues under positive selection are characterized below each schematic. There are three structures representing putative active sites (i.e., largest pockets): (B, F), sequence conservation (C, G), and sensitivity of sites to mutation (D, H).

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contained two residues under selection appeared to be moderately conserved with respect to amino acid sequence and sensitivity to mutation, and the putative active site (largest pocket, as predicted by the program fpocket) appeared to lie between the carboxyl-terminus and amine-terminus, which were predicted by Phyre2 to be in close proximity to each other (Figure 6.1 B–D). The two residues under selection within the transmembrane protein domain were not located at the active site, though one (residue 125) appeared to be within a few residues of the site (Figure 6.1 B). Amino acid sequence alignments of the *msp-1* gene at sites under selection indicated that non-synonymous mutations at sites under selection led to the following amino acid changes: 1) reside 125: A→ M, 2) residue 340: Y → T, 3) residue 1080: K → S, 4) residue 1264: R → N, 5) residue 1328: G → N (Figure 6.3).

Results from branch-site tests for *ema* identified three residues under selection (Figure 1E). The first two of these residues (residues 840, 998) reside close together in a region of the *ema* gene for which protein structure could not be predicted. The third residue (residue 1828) was located within a hydrolase domain containing an ATP-binding site (Figure 6.1 F). Amino acid conservation and sensitivity of protein structure to mutation within the hydrolase protein domain varied markedly (Figure 6.1 G, H). RaptorX analysis of the *ema* protein identified multiple hydrolase domains and an overall secondary structure composition of 49% α-helix, 11% β-sheet, and 53% loop structures, with 12% of the complete protein structure qualifying as disordered. The secondary structure of the first two residues (residues 840, 998), which were located in un-modeled regions of the protein, could not be clearly predicted by RaptorX, and were found to reside in regions of α-helix structure or disorder respectively, via Phyre2

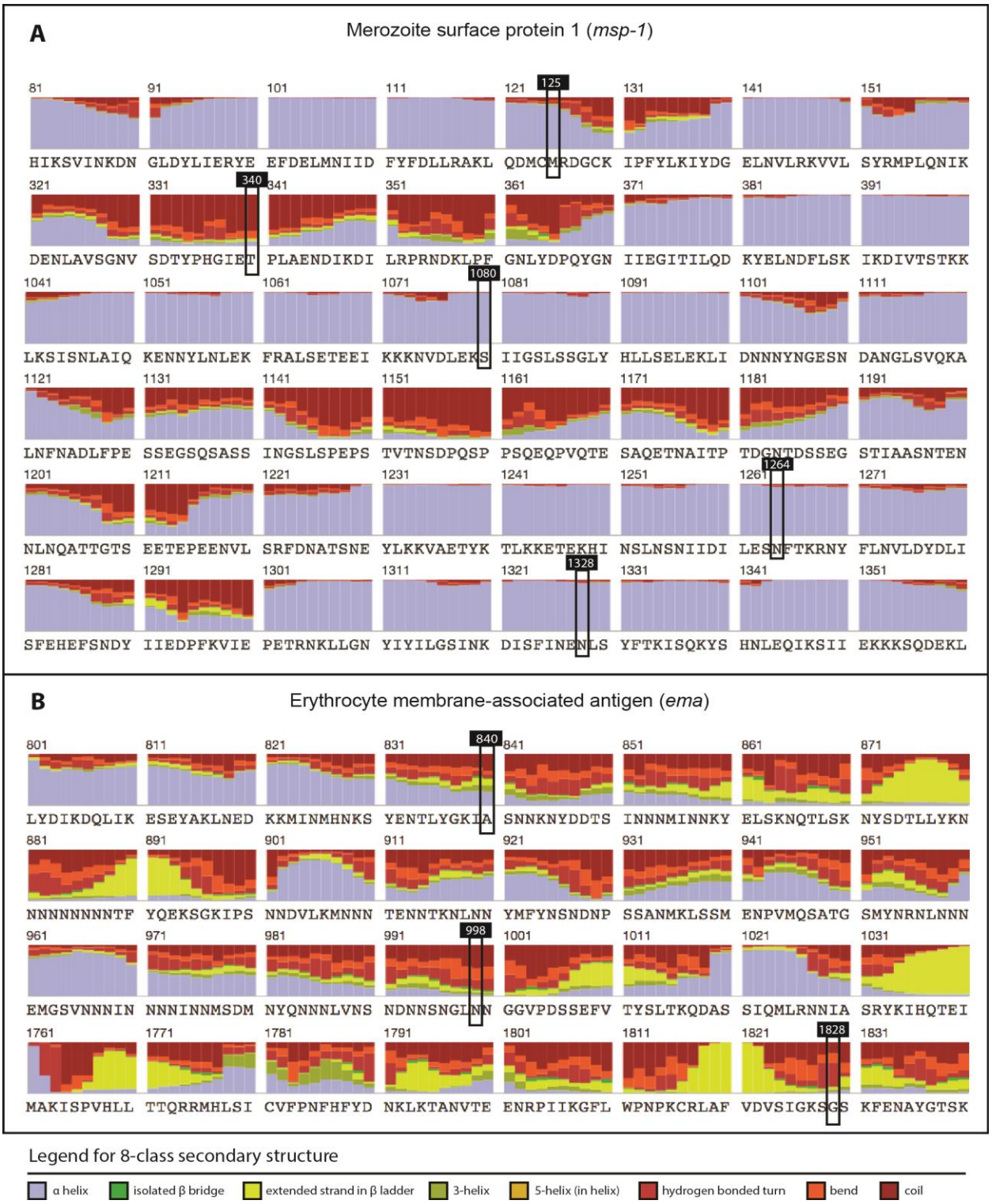

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Figure 6.2. RaptorX prediction of secondary structure (partial CDS) for A) *msp-1*, and B) *ema.* Sites under selection are indicated by black boxes; residue numbers correspond to *P. relictum* CDS.

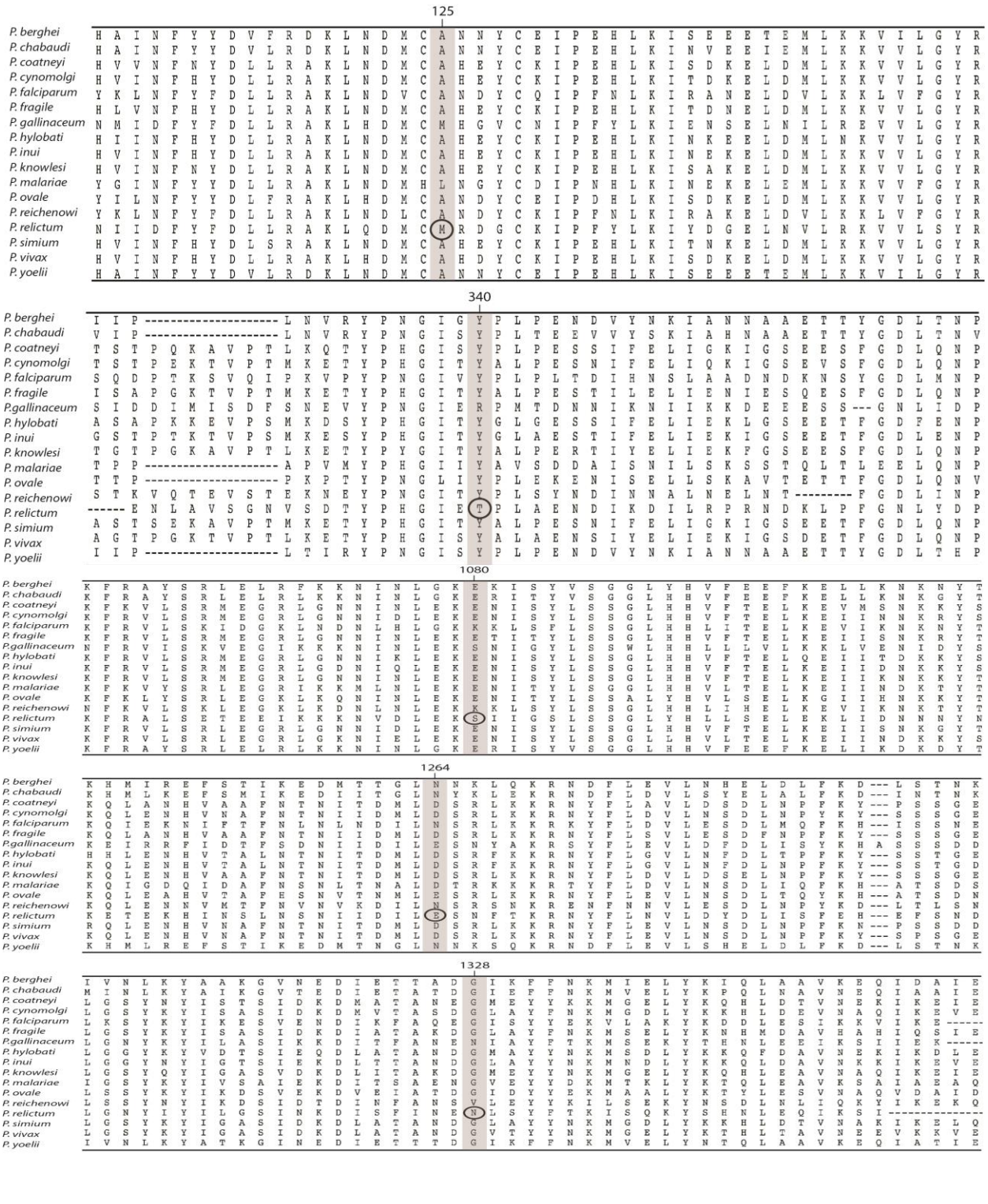

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Figure 6.3. (Previous page) Partial RevTrans amino acid sequence alignment of *msp-1* for 16 *Plasmodium* taxa. Sites under selection are circled and corresponding residues are highlighted in gray. Residue numbers correspond to the CDS of *P. relictum msp-1.*

secondary structure prediction. Amino acid sequence alignments of the *ema* gene at sites under selection indicated that non-synonymous mutations at sites under selection led to the following amino acid changes: 1) residue 840: M → A, 2) residue 998: N → L, 3) residue 1828: P → S (Figure 6.4).

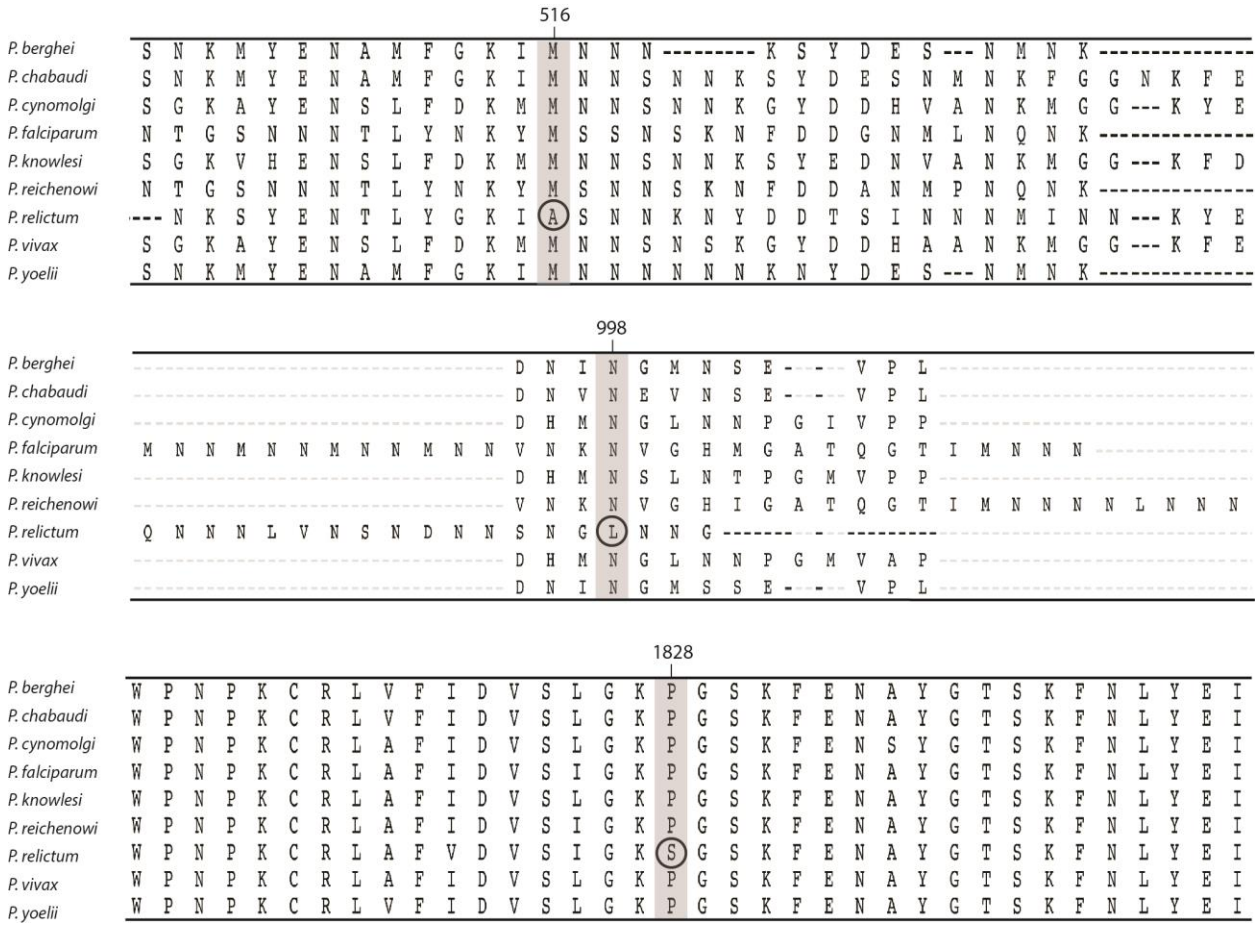

Figure 6.4. Partial RevTrans amino acid sequence alignment of *ema* for 9 *Plasmodium* taxa. Sites under selection in *P. relictum* are circled and corresponding residues are highlighted in gray. Residue numbers correspond to the CDS of *P. relictum ema.*

Analysis of msp-1$_{19}$ glyosylphosphatidylinositol-anchor domain

No residues within the highly conserved carboxyl-terminus glycosylphosphatidyl-inositol-anchor domain were found to be under significant positive selection. However,

investigation of our 16-taxon alignment revealed that 12 taxa lacked a cysteine-cysteine disulphide bond that has typically been thought to be conserved across the *Plasmodium* species (Figure 5 A). The taxa lacking this disulphide bond included all mammalian *Plasmodium* species with the exception of *P. falciparum* and *P. reichenowi*, which belong to the Laverania subgenus. The additional bond was also present in both the avian parasites *P. gallinaceum* and *P. relictum*. Phyre2 analyses of the GPI-anchor protein indicated a large putative active site, and that the protein structure has moderate to high sensitivity to amino acid mutations in general (Figure 5 B–D). Closer inspection of amino acid frequency and sensitivity to mutation revealed variation across cys-cys pairs, suggesting a slightly reduced sensitivity to mutation in the second pair of cysteine residues (out of six putatively conserved pairs), which comprise the disulphide bond missing from 12 of the taxa included in our analysis (Figure 6.6).

Discussion

Positive selection of the variable surface antigen merozoite surface protein 1 (msp-1)

Peptides encoded by the *msp-1* gene play a critical role in host red blood cell invasion by *Plasmodium* merozoites. The *msp-1* protein remains adhered to the merozoite cell membrane via a glyosylphosphatidylinositol (GPI) anchor encoded by the p19 peptide (hereafter *msp-1*$_{19}$) near the protein C-terminus. Through successive rounds of cleavage of *msp-1*, the *msp-1*$_{19}$ sub-unit remains anchored to the surface of the merozoite cell membrane via the GPI anchor located at the carboxyl-terminus of the protein (Kadekoppala and Holder 2010). Because of its significant role in initial host cell invasion and persistence of infection, we sought to identify regions of the

msp-1 gene that are under positive Darwinian selection. Specifically, we explored selection of this gene in the avian parasite *P. relictum*, which has a broad geographic distribution and host range (Valkiūnas 2005), and thus is likely to experience an unusual number of selection pressures throughout its distribution. Tests for selection identified five individual amino acid residues in the *msp-1* gene that appear to be under significant positive selection (Figure 6.1 A). Of these five residues, two were located near the armine-terminus of *msp-1* (residues 125 and 340) within a transmembrane protein domain. Although neither residue was located within the putative active site of the peptide (Fig 6.5 B), it is possible that the non-synonymous mutations at these sites have elicited a significant

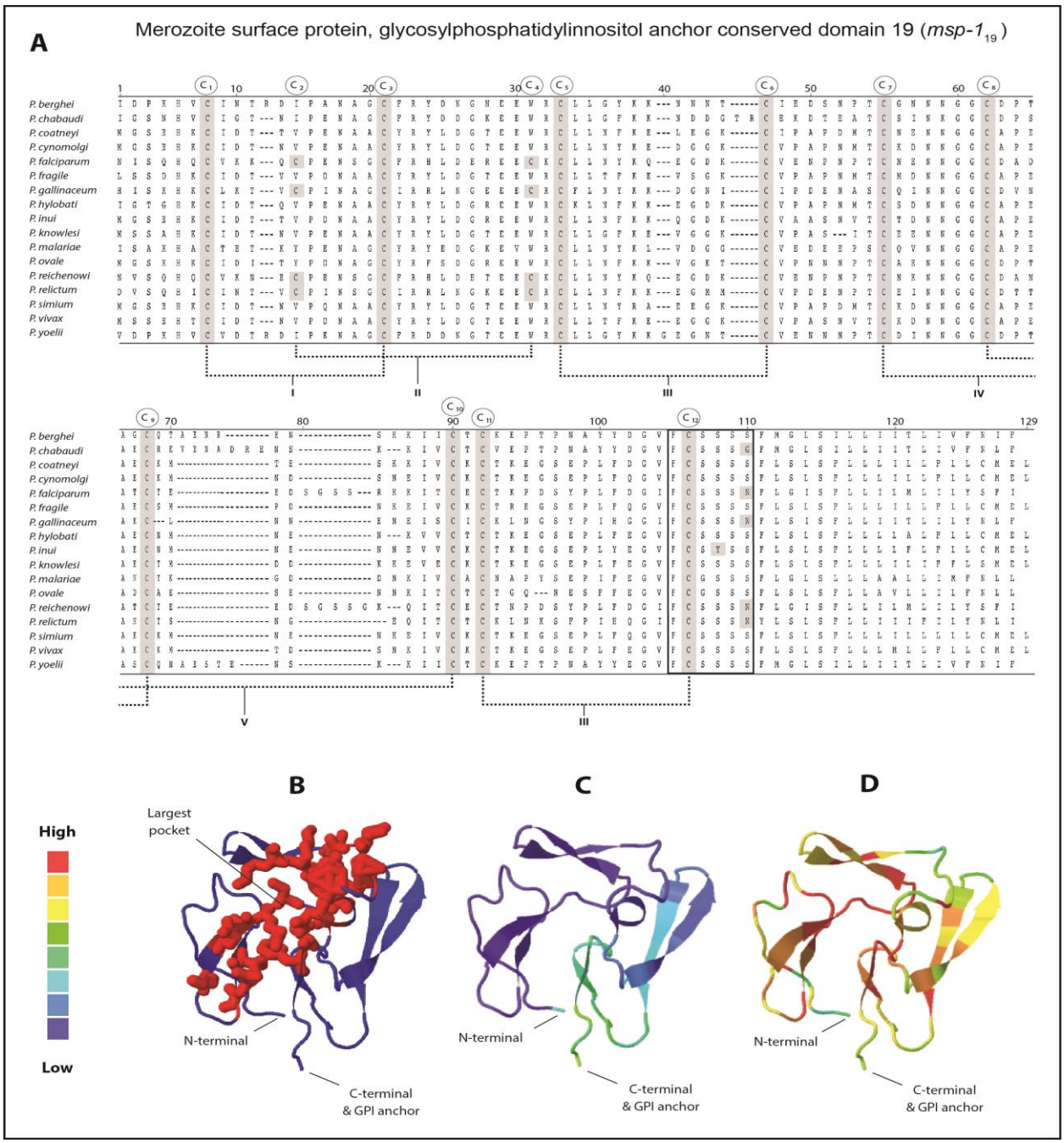

Figure 6.5. A) Amino acid sequence alignment of the *msp-1*$_{19}$ peptide from 17 Plasmodium sequences (representing avian, primate, and rodent lineages). Cysteine residues are highly conserved, with the exception of residues C2 and C4, suggesting the absence of a disulphide bond and thus, difference in protein structure, for species with alternative amino acids at these sites. The glycosylphosphatidylinnositol (GPI) anchor, identified by the generally conserved "FCSSS" amino acid sequence motif (open box) also exhibits mutations within the last two serine residues for some species. There are three structures representing B) putative active sites (i.e., largest pockets), C),sequence conservation, and D) sensitivity of sites to mutation.

change in the form and/or function of the protein. Similarly, the effects of the remaining three

residues (residues 1080, 1264, 1328) are unclear. These residues reside closer to the carboxyl-terminus at sites for which only structure could be determined. Secondary structure analyses indicated that these three residues are found on α-helices (the dominant secondary structure of the *msp-1* protein, according to our analyses) (Fig 6.2 A).

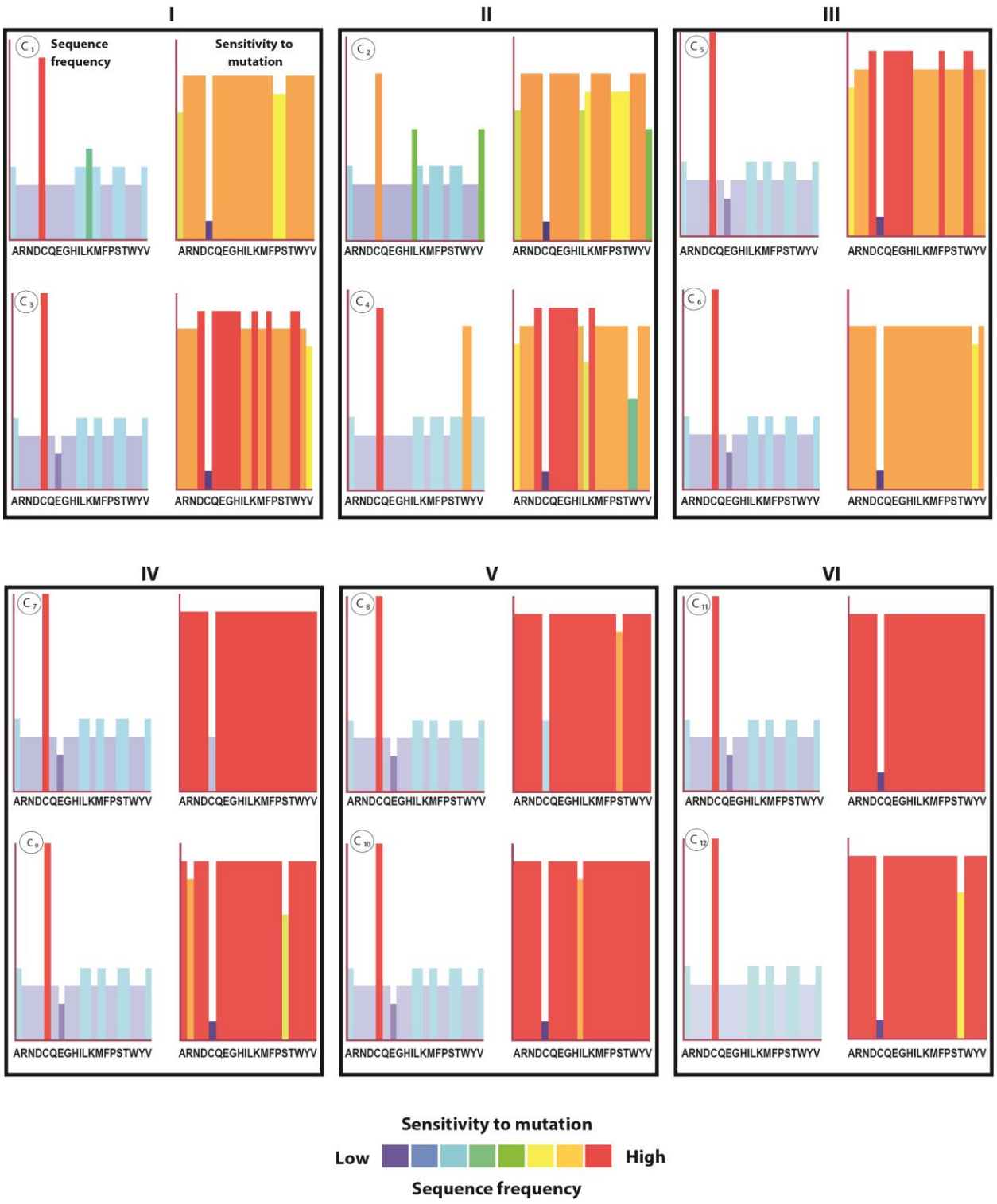

Figure 6.6. Sequence frequency and sensitivity to mutation of Cysteine sites to amino acid mutations located on the *msp-1*$_{19}$ peptide, based on SuSPect analysis of canonical Plasmodium *msp-1* sequence (*P. falciparum*). Bold numbered boxes correspond to cys-cys disulphide bond pairs, and individual cysteine residues are labeled with numbered circles (see corresponding Figure 6.5).

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A recent study of the *msp-1* gene in *P. relictum* (SGS1) found a high level of conservation *msp-1*$_{19}$ cysteine motifs between *P. relictum*, *P. gallinaceum*, and *P. falciparum*. The cysteine (cys) residues are connected by disulphide bonds that produce a relatively conserved structure similar to epidermal growth factor–like domains (EGF) (Babon et al. 2007; Hellgren et al. 2013) (Figure 6.5). We found general conservation of cys residues, as well as conservation of the "FCSSS" motif characteristic of the *msp-1*$_{19}$ GPI anchor in our comparison of 17 *Plasmodium* species. However, there were several exceptions. Interestingly, one of the six cysteine pairs identified as conserved in *P. relictum*, *P. gallinaceum*, and *P. falciparum* (Hellgren et al. 2013) is shared only by *P. reichenowi*, and is not conserved in the 13 other Plasmodium species included in our analysis (Figure 6.5 A).

Although these sites were not identified as being under selection, the absence of the cys-cys disulphide bond is likely to have an effect on the secondary structure of the p19 peptide in certain species. The presence of an additional cys-cys disulphide bond in *P. relictum*, *P. gallinaceum*, *P. falciparum*, and *P. reichenowi* species, which do not form a monophyletic group (Lutz et al. 2016), has either arisen through convergent evolution, or is an ancestral trait that has subsequently been lost in the majority of mammalian parasite lineages. Sequencing of additional avian and mammalian *Plasmodium* species will be necessary to determine which scenario is most likely. The inclusion of *msp-1* sequences from chiropteran *Nycteria* and *Polychromophilus* parasites should be particularly informative, as these genera are sister to all other mammalian *Plasmodium* parasites, and share a most recent common ancestor with avian *Plasmodium* (Lutz et al. 2016). It seems likely, given the phylogenetic relationships of the 16 taxa included in our *msp-1* analyses, that the additional disulphide bond was subsequently lost in the non-Laverania *Plasmodium* parasites of mammals (Figure 6.7).

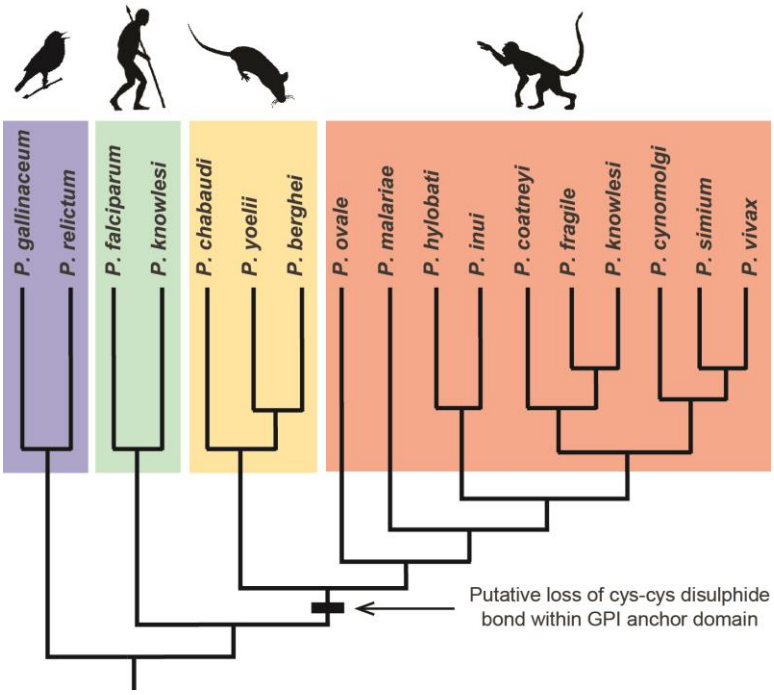

Figure 6.7. Cladogram showing relationships of *Plasmodium* taxa included in *msp-1* analyses (based on the phylogeny of Lutz et al. 2016). Host–parasite relationships are indicated with colored boxes (blue = avian hosts; green = great ape hosts; yellow = rodent hosts; orange = primate hosts). The putative loss of a cys-cys disulphide bond in the *msp-1*$_{19}$ domain is indicated by a dash and arrow on the cladogram.

3375

3376 The phenotypic effect of an additional cys-cys disulphide bond is unclear, but it is

3377 interesting to note that the species in which it is present exhibit either high levels of

3378 pathogenicity and cerebral malaria [*P. falciparum* (Taylor et al. 2012), *P. gallinaceum* (de Matos

3379 Macchi et al. 2010), *P. reichenowi* (Herbert et al. 2015)], or are able to infect a very broad range

3380 of hosts [*P. relictum* (Valkiūnas 2005)]. The *msp-1*$_{19}$ peptide remains embedded in the merozoite

3381 surface membrane for the duration of parasitic infection (Kadekoppala and Holder 2010), and is

3382 known to elicit a strong immunological response in host organisms (Changrob et al. 2015;

3383 Siddiqui et al. 1987), making it a compelling candidate for vaccine development (Curd et al.

3384 2014; Faber et al. 2007; Holder 2009). Further study of the effects of mutations that lead to

phenotypic changes, such as loss or gain of disulphide bonds, will likely be important for the development of antimalarial drugs that target epitopes like the *msp-1*$_{19}$ peptide.

Positive selection of the erythrocyte membrane-associated antigen (ema)

Of the three amino acid sites predicted to under selection in the *ema* gene, one is of particular interest. Residue 1828 (Fig 6.1 E–H) is located within a hydrolase domain, and although this residue does not appear to be tightly associated with the putative active site (largest pocket) or ATP binding site, it is possible that a mutation at this site may have important functional implications for the hydrolase molecule. Hydrolases are hydrolytic enzymes that are responsible for carrying out roles that are crucial for the organism's survival, primarily by cleaving chemical bonds using water. *Plasmodium* parasites are eukaryotic and therefore rely on many hydrolytic pathways similar to those of their hosts, such as the ubiquitin-proteasome system, for survival (Artavanis-Tsakonas et al. 2010). Non-synonymous substitutions within a hydrolase domain may alter or enhance the ability of the enzyme to bind to other molecules, and such may be the case for sites under selection in the *ema* gene. Currently, very little is known about *emp* proteins in non-model *Plasmodium* parasites, and the inability of Phyre2 to predict a more detailed protein structure for the additional two residues under selection limit our ability to assess how non-synonymous substitutions at residues 840 and 998 might affect the function of *emp*-encoded proteins. Both Phyre2 and RaptorX analyses of the secondary structure at these sites indicate that they are quite disordered. Although the secondary structure of disordered regions cannot be readily predicted, they often serve functionally important roles in proteins

(Dunker et al. 2008; Xie et al. 2007). However, it is nearly impossible to predict the effects of mutations on those roles.

Conclusion

Continued proteomic studies of *Plasmodium* proteins, and in particular those proteins involved with host cell invasion and parasite persistence within the host cell, will play an important role in elucidating the evolutionary history of Plasmodium parasites. Combining molecular data with proteomic data will ultimately allow us to more closely study cases of adaptive evolution by identifying amino acid sites that are under selection and then assessing how mutations at those sites influence the structure and function of proteins that play an important role in the life history and persistence of Plasmodium parasites. In addition to improving our knowledge of protein structure and function, the inclusion of more non-model taxa in comparative genomic analyses will lend more power to tests of positive selection, and thus reduce spurious results and improve our ability to accurately identify amino acid sites that are truly under selection.

ACKNOWLEDGMENTS

This project was supported in part by the Field Museum of Natural History's Emerging Pathogens Project, funded by the Davee Foundation and the Dr. Ralph and Mariam Faulk Medical Research Trust, and in part by grants from the Athena Fund at the Cornell Lab of Ornithology. Support from the National Science Foundation Malaria Research Coordination Network also aided in fostering and maintaining the collaborations required during this project. We also thank the Sanger Institute for their provision of unpublished sequence data from *Plasmodium relictum*.

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APPENDIX A

GIEMSA STAINING PROTOCOL FOR HAEMATOZOAN PARASITES

1.Prepare alkaline (N_2HPO_4) and acid (KH_2PO_4) stock phosphate buffers as follows:

- Buffer A: (9.50 g N_2HPO_4) + (990.50 mL dH2O) = 1000 mL Alkaline stock
- Buffer B: (9.07g KH_2PO_4) + (990.93 mL dH2O) = 1000 mL Acid stock
- Working Buffer: (61 mL Buffer A) + (39 mL Buffer B) = 100 mL Working buffer
  The working buffer pH should be in the range 7.0 – 7.2

Stock buffers can be kept and reused to prepare a working buffer, which should be made fresh every few days. Stock buffers can be stored at room temperature indefinitely. Fresh working buffer should be made every few days, and can be stored at room temperature as well.

2. Place a thin layer of high quality Giemsa stain at the bottom of a Coplin jar, and add working buffer to produce a ~10% buffered Giemsa stain

3. Slides should have already been fixed in methanol after blood smear preparation (in the field). However, it is a good idea to dip slides in methanol again before staining, particularly if they have been exposed to humidity, dust, etc. This additional methanol rinse will produce cleaner and more evenly stained slides.

3. Add slides to Coplin jar and allow to stain for 60-90 minutes. The duration of staining time will vary depending on the age of the slides, the quality of the Giemsa stain, and the concentration of the buffered Giemsa stain. Older slides tend to take up stain more readily, and are likely to stain too darkly of left for too long. It is a good idea to test one or two slides before processing an entire batch.

4. Once staining is complete, remove slides from Coplin jar and rinse residual stain off under water.

5. Slides should be labeled archivally, and label should at minimum include the host voucher number. Once dry, slides should be placed in a secure slide box for long term storage.

APPENDIX B

ALUM CARMINE STAINING PROTOCOL FOR ENDOPARASITES

1. Rinse worms in distilled water. Rinse time depends on the size of the specimens. Water may need to be changed once for larger specimens. When transferred from ethanol to water specimens will float. Sink them by pipetting water onto them or by using a soft tool such as a paintbrush.

2. Transfer specimens to stain. The stock solution of the stain can be diluted with distilled water immediately prior to staining. The level of dilution is flexible, but a stain that is roughly 2x diluted usually works wellTest stain a few specimens of lesser value and you will know what works best. Staining time can be from a few minutes for small specimens with concentrated carmine to more than 30 minutes for large worms using more diluted stain.

3. Transfer specimens to water to rinse off the stain.

4. De-stain in acid alcohol (0.5-1% solution of HCl in 70% ethanol) while observing the specimen under dissecting scope. Replenish the acid alcohol if it becomes too pink. De-staining may take only seconds in some cases, therefore we recommend using 0.5% HCl solution in ethanol to avoid rapid destaining.

5. Transfer specimens to water to rinse off the de-staining solution. At this point specimens can be straightened if needed. This can be usually achieved by stretching specimens on a piece of paper while keeping them under a thin layer of water, then add 70% ethanol and keep adding ethanol in small portions (to keep specimen wet at all times) until the specimen is hardened and can be transferred into a beaker for further dehydration.

6. Dehydration. Specimens need to be moved through a series of ethanols of ascending concentration. Ethanols at 50%, 70%, 80%, 90%, 100% are recommended (95% can be added between 90% and 100%) ethanol. To ensure complete dehydration an additional change of 100% ethanol is recommended. Make sure that specimens are not exposed to air at any point during the procedure to avoid an immediate desiccation and loss of the specimens. Time in each ethanol depends on the size of specimens; 30 minutes in each grade is usually sufficient for small specimens up to 3-4 mm in length. Longer times are recommended for larger/thicker specimens. An hour is recommended in 100% ethanol.

7. Clearing. After water have been removed from the specimens by dehydration, they are transferred to a clearing agent (clove oil [eugenol] is recommended). The clearing agent renders the parasite transparent and is miscible with the mounting medium of choice.

8. Mounting. We strongly recommend Damar Gum as the embedding medium. It is sold by many suppliers and is clear, cheap, relatively fast drying (much faster than Canada balsam), and xylene soluble so can be re-mounted if needed. The embedding medium hardens as the solvent evaporates, making a permanent mount of your specimen. To provide a support for specimens we use pre-cut pieces of cover slips placed on both sides of a specimen prior to covering it with cover slip.

APPENDIX C

MAYER'S HAEMATOXYLIN STAINING PROTOCOL FOR ENDOPARASITES (SIMILAR TO ALUM CARMINE PROTOCOL, WITH A FEW NOTABLE DIFFERENCES)

1. Rinse worms in distilled water as with alum carmine protocol.

2. Transfer specimens to stain. The stock solution of the stain needs to be diluted at least 1:1 with distilled water. Do not use metal instruments when working with haematoxylin. Use only a dedicated pipette to not mix with other chemicals. Somewhat longer staining time with more diluted haematoxyline usually produces better results, but it depends on the group of parasites, fixation, etc. Usually staining takes from 15 to 60 minutes.

3. Transfer specimens to water to rinse off the stain.

4. De-staining in 1% aqueous solution of HCl while observing the specimen under a dissecting scope. The body filling tissue (parenchyma) should be free of stain, but enough stain should remain to color the internal organs. Large specimens with thick tegument may not be transparent enough for good assessment of coloration. In these cases one has to rely on experience.

5. Transfer specimens to 1% ammonia solution to neutralize de-staining process. Coloration will change from red to blue or purple. Use water to rinse off the de-staining solution. At this point specimens can be straightened if needed (see above).

6. Dehydration. Specimens need to be moved through a series of ethanols of ascending concentration as above.

7. Clearing. After water has been removed from the specimens by dehydration, they are transferred to a clearing agent. In this case either methyl salicylate or clove oil can be used. Methyl salicylate usually produces somewhat more contrasting coloration. Specimens should be first transferred to methyl salicylate/ethanol mix in 1:1 ration and then to pure methyl salicylate. See above for directions on using Clove oil.

8. Mount specimens as above.

APPENDIX D

PRIMERS AND THERMAL CYCLING CONDITIONS

Protocol / Parasite genera	Primer	Primer Sequence	Thermal Cycling Conditions*
Nested PCR to amplify 479 bp of *Plasmodium* and *Haemoproteus* spp. cytochrome *b*			
Primer pair 1	HAEMNF[a]	5' - CATATATTAAGAGAATTATGGAG - 3'	[94/30, 50/30, 72/45] x 20
	HAEMNR2[a]	5' - AGAGGTGTAGCATATCTATCTAC - 3'	
Primer pair 2	HAEMF[b]	5' - ATGGTGCTTTCGATATATGCATG - 3'	[94/30, 50/30, 72/45] x 35
	HAEMR2[b]	5' - GCATTATCTGGATGTGATAATGGT - 3'	
Nested PCR to amplify 479 - 526 bp of *Leucotozoons* spp. cytochrome *b*			
Primer pair 1	HAEMNFI[c]	5' - CATATATTAAGAGAAITATGGAG - 3'	[94/30, 51/30, 72/45] x 20
	HAEMNR3[c]	5' - ATAGAAAGATAAGAAATACCATTC - 3'	
Primer pair 2	HAEMFL[c]	5' - ATGGTGTTTTAGATACTTACATT - 3'	[94/30, 51/30, 72/45] x 35
	HAEMR2L[c]	5' - CATTATCTGGATGAGATAATGGIGC - 3'	
Primer pair 2 (modified)	L350F[d]	5' - GGTGTTTTAGATACTTA -3'	[94/30, 51/30, 72/45] x 35
	L890R[d]	5' - TACAATATGTTGAGGTGTTTG - 3'	
Sequencing primers (modified)	L545F[d]	5' - ACAAATGAGTTTCTGGGGA - 3'	
	L825R[d]	5' - GCAATTCCAAATAAACTTTGAA - 3'	

* Temperature (˚C)/time (s) for denaturation, annealing, and extension steps; outer thermal cycling conditions included an initial denaturation period of 94˚C for 3 minutes and a final extension period of 72˚C for 10 minutes

[a] Waldenström et al., 2004
[b] Bensch et al., 2000
[c] Hellgren et al., 2004
[d] This study

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Host Collection Number	Field Site*	Host Order	Host Family	Host Genus	Host species	Host subspecies	Host common name	Parasite name	Lineage name**	Parasite GenBank Accession Number
467839	1	Anseriformes	Anatidae	*Alopochen*	*aegyptiacus*		Egyptian Goose	0	NA	NA
467841	1	Anseriformes	Anatidae	*Sarkidiornis*	*melanotos*	*melanotos*	Knob-billed Duck	0	NA	NA
467840	1	Anseriformes	Anatidae	*Sarkidiornis*	*melanotos*	*melanotos*	Knob-billed Duck	*Plasmodium* sp.	P_BUL07	KM056642
467918	2	Bucerotiformes	Bucerotidae	*Tockus*	*alboterminatus*	*suahelicus*	Crowned Hornbill	*Leucocytozoon* sp.	L_AFR207	KM056525
467918	2	Bucerotiformes	Bucerotidae	*Tockus*	*alboterminatus*	*suahelicus*	Crowned Hornbill	*Plasmodium* sp.	P_ACCTAC01	KM056621
467919	1	Bucerotiformes	Bucerotidae	*Tockus*	*nasutus*	*caffer*	African Grey Hornbill	*Leucocytozoon* sp.	L_AFR209	KM056527
467886	1	Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*pectoralis*	*fervidus*	Fiery-necked Nightjar	0	NA	NA
467887	1	Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*pectoralis*	*fervidus*	Fiery-necked Nightjar	0	NA	NA
467888	2	Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*poliocephalus*	*guttifer*	Ruwenzori Nightjar	0	NA	NA
467889	2	Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*poliocephalus*	*guttifer*	Ruwenzori Nightjar	0	NA	NA
467890	2	Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*poliocephalus*	*guttifer*	Ruwenzori Nightjar	0	NA	NA
467892	1	Caprimulgiformes	Caprimulgidae	*Scotornis*	*fossii*	*welwitschii*	Square-tailed Nightjar	0	NA	NA
467893	1	Caprimulgiformes	Caprimulgidae	*Scotornis*	*fossii*	*welwitschii*	Square-tailed Nightjar	0	NA	NA
467834	NA	Ciconiiformes	Ardeidae	*Ardea*	*melanocephala*		Black-headed Heron	*Parahaemoproteus* sp.	H_QUERY01	KM056416
467835	NA	Ciconiiformes	Ardeidae	*Bubulcus*	*ibis*		Cattle Egret	Unknown	Coinfection	NA
467836	NA	Ciconiiformes	Ardeidae	*Bubulcus*	*ibis*		Cattle Egret	*Parahaemoproteus* sp.	H_QUERY01	KM056416
467837	NA	Ciconiiformes	Scopidae	*Scopus*	*umbretta*		Hamerkop	*Parahaemoproteus* sp.	H_QUERY01	KM056416
467837	NA	Ciconiiformes	Scopidae	*Scopus*	*umbretta*		Hamerkop	*Leucocytozoon* sp.	L_AFR210	KM056528
467894	1	Coliiformes	Coliidae	*Colius*	*striatus*	*berlepschi*	Speckled Mousebird	0	NA	NA
467895	1	Coliiformes	Coliidae	*Colius*	*striatus*	*berlepschi*	Speckled Mousebird	*Plasmodium* sp.	P_AFR33	KM056595
467865	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	Unknown	Coinfection	NA
467866	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	Unknown	Coinfection	NA
467865	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	*Haemoproteus* sp.	H_AFR112	KM056423
467866	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	*Haemoproteus* sp.	H_AFR119	KM056425
467867	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	*Parahaemoproteus* sp.	H_AFR120	KM056426
467867	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	*Leucocytozoon* sp.	L_AFR178	KM056496
467867	2	Columbiformes	Columbidae	*Columba*	*arquatrix*		African Olive-Pigeon	*Leucocytozoon* sp.	L_AFR220	KM056535
467860	1	Columbiformes	Columbidae	*Streptopelia*	*capicola*	*tropica*	Cape Turtle Dove	0	NA	NA
467861	1	Columbiformes	Columbidae	*Streptopelia*	*capicola*	*tropica*	Cape Turtle Dove	0	NA	NA
467862	2	Columbiformes	Columbidae	*Streptopelia*	*semitorquata*	*semitorquata*	Red-eyed Dove	*Haemoproteus* sp.	H_AFR109	KM056421
467868	1	Columbiformes	Columbidae	*Treron*	*calva*	*schalowi*	African Green-Pigeon	*Haemoproteus* sp.	H_AFR70	KM056461
467870	1	Columbiformes	Columbidae	*Turtur*	*chalcospilos*	*chalcospilos*	Emerald-spotted Wood-Dove	*Haemoproteus* sp.	H_AFR44	KM056450
467871	1	Columbiformes	Columbidae	*Turtur*	*chalcospilos*	*chalcospilos*	Emerald-spotted Wood-Dove	*Haemoproteus* sp.	H_AFR44	KM056450
467869	1	Columbiformes	Columbidae	*Turtur*	*chalcospilos*	*chalcospilos*	Emerald-spotted Wood-Dove	0	NA	NA
467901	1	Coraciiformes	Alcedinidae	*Alcedo*	*cristata*	*cristata*	Malachite Kingfisher	0	NA	NA
467900	1	Coraciiformes	Alcedinidae	*Halcyon*	*senegalensis*	*cyanoleuca*	Woodland Kingfisher	*Parahaemoproteus* sp.	H_AFR151	KM056440
467900	1	Coraciiformes	Alcedinidae	*Halcyon*	*senegalensis*	*cyanoleuca*	Woodland Kingfisher	*Leucocytozoon* sp.	L_AFR208	KM056526
467905	1	Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	Unknown	Coinfection	NA
467903	1	Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	*Parahaemoproteus* sp.	H_AFR67	KM056460
467902	1	Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	*Haemoproteus belopolskyi*	H_SW1	KM056409
467902	1	Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	*Plasmodium* sp.	P_AFR46	KM056598
467902	1	Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	*Plasmodium* sp.	P_AFR6	KM056605
467917	1	Coraciiformes	Coraciidae	*Eurystomus*	*glaucurus*		Broad-billed Roller	*Leucocytozoon* sp.	L_AFR162	KM056481
467915	1	Coraciiformes	Meropidae	*Merops*	*apiaster*		European Bee-eater	*Parahaemoproteus* sp.	H_QUERY01	KM056416
467915	1	Coraciiformes	Meropidae	*Merops*	*apiaster*		European Bee-eater	*Leucocytozoon* sp.	L_AFR251	KM056562
467914	1	Coraciiformes	Meropidae	*Merops*	*apiaster*		European Bee-eater	0	NA	NA
467909	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	*Parahaemoproteus* sp.	H_AFR35	KM056447
467911	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	*Parahaemoproteus* sp.	H_AFR35	KM056447
467910	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	0	NA	NA

467912	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	0	NA	NA
467913	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	0	NA	NA
467909	1	Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	*Plasmodium* sp.	P_BUL07	KM056642
467877	1	Cuculiformes	Cuculidae	*Centropus*	*superciliosus*	*loandae*	White-browed Coucal	*Parahaemoproteus* sp.	H_AFR61	KM056457
467876	1	Cuculiformes	Cuculidae	*Centropus*	*superciliosus*	*loandae*	White-browed Coucal	*Plasmodium* sp.	P_BUL07	KM056642
467882	2	Cuculiformes	Cuculidae	*Chrysococcyx*	*klaas*	*klaas*	Klaas's Cuckoo	*Parahaemoproteus* sp.	H_AFR8	KM056468
467878	1	Cuculiformes	Cuculidae	*Cuculus*	*canorus*	*gularis*	African Cuckoo	0	NA	NA
467842	1	Falconiformes	Accipitridae	*Aquila*	*wahlbergi*		Wahlberg's Eagle	*Plasmodium* sp.	P_RTSR1	KM056623
467843	1	Falconiformes	Accipitridae	*Milvus*	*migrans*	*parasitus*	Yellow-billed Kite	*Parahaemoproteus* sp.	H_AFR48	KM056451
467850	1	Galliformes	Numididae	*Numida*	*meleagris*	*mitrata*	Helmeted Guineafowl	*Parahaemoproteus* sp.	H_AFR50	KM056452
467848	2	Galliformes	Phasianidae	*Coturnix*	*coturnix*	*africana*	Common Quail	0	NA	NA
467844	2	Galliformes	Phasianidae	*Francolinus*	*levaillantii*	*crawshayi*	Red-winged Francolin	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
467845	2	Galliformes	Phasianidae	*Francolinus*	*levaillantii*	*crawshayi*	Red-winged Francolin	*Leucocytozoon* sp.	L_AFR245	KM056558
467845	2	Galliformes	Phasianidae	*Francolinus*	*levaillantii*	*crawshayi*	Red-winged Francolin	*Plasmodium* sp.	P_BUL07	KM056642
468628	2	Galliformes	Phasianidae	*Gallus*	*gallus*		Chicken	*Parahaemoproteus* sp.	H_QUERY01	KM056416
468628	2	Galliformes	Phasianidae	*Gallus*	*gallus*		Chicken	*Leucocytozoon schoutedeni*	L_GALLUS06	KM056646
467880	1	Galliformes	Phasianidae	*Gallus*	*gallus*		Chicken	0	NA	NA
468623	1	Galliformes	Phasianidae	*Gallus*	*gallus*		Chicken	0	NA	NA
467853	2	Gruiformes	Rallidae	*Sarothrura*	*rufa*	*rufa*	Red-chested Flufftail	*Plasmodium* sp.	P_AFR118	KM056573
467853	2	Gruiformes	Rallidae	*Sarothrura*	*rufa*	*rufa*	Red-chested Flufftail	*Plasmodium* sp.	P_PSEGRI01	KM056637
467874	2	Musophagiformes	Musophagidae	*Tauraco*	*corythaix*	*schalowi*	Schalow's Turaco	Unknown	Coinfection	NA
467874	2	Musophagiformes	Musophagidae	*Tauraco*	*corythaix*	*schalowi*	Schalow's Turaco	*Parahaemoproteus* sp.	H_AFR59	KM056456
467947	1	Passeriformes	Alaudidae	*Mirafra*	*rufocinnamomea*	*fischeri*	Flappet Lark	0	NA	NA
467946	1	Passeriformes	Alaudidae	*Mirafra*	*rufocinnamomea*	*fischeri*	Flappet Lark	*Plasmodium* sp.	P_ACCTAC01	KM056621
467945	1	Passeriformes	Alaudidae	*Mirafra*	*rufocinnamomea*	*fischeri*	Flappet Lark	*Plasmodium* sp.	P_AFR69	KM056609
468254	1	Passeriformes	Cisticolidae	*Apalis*	*thoracica*	*youngi*	Bar-throated Apalis	*Leucocytozoon* sp.	L_AFR181	KM056499
468254	1	Passeriformes	Cisticolidae	*Apalis*	*thoracica*	*youngi*	Bar-throated Apalis	*Leucocytozoon* sp.	L_AFR182	KM056500
468251	2	Passeriformes	Cisticolidae	*Apalis*	*thoracica*	*youngi*	Bar-throated Apalis	0	NA	NA
468253	2	Passeriformes	Cisticolidae	*Apalis*	*thoracica*	*youngi*	Bar-throated Apalis	0	NA	NA
468260	1	Passeriformes	Cisticolidae	*Calamonastes*	*stierlingi*	*irwini*	Stierling's Wren-Warbler	*Leucocytozoon* sp.	L_AFR218	KM056533
468258	1	Passeriformes	Cisticolidae	*Calamonastes*	*stierlingi*	*irwini*	Stierling's Wren-Warbler	*Plasmodium* sp.	P_RFF1	KM056632
468260	1	Passeriformes	Cisticolidae	*Calamonastes*	*stierlingi*	*irwini*	Stierling's Wren-Warbler	*Plasmodium* sp.	P_WW4	KM056626
468204	1	Passeriformes	Cisticolidae	*Cisticola*	*brachyptera*	*isabellina*	Short-winged Cisticola	*Leucocytozoon* sp.	L_AFR211	KM056529
468204	1	Passeriformes	Cisticolidae	*Cisticola*	*brachyptera*	*isabellina*	Short-winged Cisticola	*Plasmodium* sp.	P_AFR5	KM056601
468205	1	Passeriformes	Cisticolidae	*Cisticola*	*brachyptera*	*isabellina*	Short-winged Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468232	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	Unknown	Coinfection	NA
468236	2	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	Unknown	Coinfection	NA
468234	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	0	NA	NA
468235	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	0	NA	NA
468225	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	*Plasmodium* sp.	P_AFR13	KM056579
468227	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	*Plasmodium* sp.	P_AFR26	KM056592
468226	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	*Plasmodium* sp.	P_PSEGRI01	KM056637
468233	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	*Plasmodium* sp.	P_PSEGRI01	KM056637
468228	1	Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468237	1	Passeriformes	Cisticolidae	*Cisticola*	*fulvicapilla*	*muelleri*	Piping Cisticola	*Haemoproteus lanii*	H_RBS4	KM056411
468220	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Parahaemoproteus* sp.	H_AFR34	KM056446
468216	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Plasmodium* sp.	P_AFR13	KM056579
468220	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Plasmodium* sp.	P_AFR13	KM056579
468221	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Plasmodium* sp.	P_AFR37	KM056596
468224	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Plasmodium* sp.	P_LINOLI01	KM056629
468215	1	Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468192	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468298	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	Unknown	Coinfection	NA

468190	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Haemoproteus payevski*	H_RW1	KM056406
468193	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Leucocytozoon* sp.	L_AFR173	KM056491
468195	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Leucocytozoon* sp.	L_AFR211	KM056529
468188	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	0	NA	NA
468189	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	0	NA	NA
468191	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Plasmodium* sp.	P_AFR105	KM056566
468192	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Plasmodium* sp.	P_AFR106	KM056567
468194	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Plasmodium* sp.	P_AFR107	KM056568
468190	2	Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	*Plasmodium* sp.	P_PSEGRI01	KM056637
468201	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	*Parahaemoproteus* sp.	H_AFR103	KM056420
468202	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	*Leucocytozoon* sp.	L_AFR211	KM056529
468196	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	0	NA	NA
468200	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	0	NA	NA
468197	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	*Plasmodium* sp.	P_PSEGRI01	KM056637
468202	2	Passeriformes	Cisticolidae	*Cisticola*	*njombe*		Churring Cisticola	*Plasmodium* sp.	P_PSEGRI01	KM056637
468210	1	Passeriformes	Cisticolidae	*Cisticola*	*rufilata*	*ansorgei*	Grey Cisticola	*Plasmodium* sp.	P_AFR13	KM056579
468207	1	Passeriformes	Cisticolidae	*Cisticola*	*rufilata*	*ansorgei*	Grey Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468208	1	Passeriformes	Cisticolidae	*Cisticola*	*rufilata*	*ansorgei*	Grey Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468209	1	Passeriformes	Cisticolidae	*Cisticola*	*rufilata*	*ansorgei*	Grey Cisticola	*Plasmodium* sp.	P_RFF1	KM056632
468214	2	Passeriformes	Cisticolidae	*Cisticola*	*woosnami*	*lufira*	Trilling Cisticola	Unknown	Coinfection	NA
468212	2	Passeriformes	Cisticolidae	*Cisticola*	*woosnami*	*lufira*	Trilling Cisticola	0	NA	NA
468213	2	Passeriformes	Cisticolidae	*Cisticola*	*woosnami*	*lufira*	Trilling Cisticola	*Plasmodium* sp.	P_AFR143	KM056585
468211	2	Passeriformes	Cisticolidae	*Cisticola*	*woosnami*	*lufira*	Trilling Cisticola	*Plasmodium* sp.	P_WW4	KM056626
468176	1	Passeriformes	Cisticolidae	*Prinia*	*erythroptera*	*rhodoptera*	Red-winged Warbler	*Leucocytozoon* sp.	L_AFR218	KM056533
468176	1	Passeriformes	Cisticolidae	*Prinia*	*erythroptera*	*rhodoptera*	Red-winged Warbler	*Plasmodium* sp.	P_SYBOR11	KM056638
468625	2	Passeriformes	Corvidae	*Corvus*	*albicollis*		White-necked Raven	*Parahaemoproteus* sp.	H_AFR2	KM056443
468627	2	Passeriformes	Corvidae	*Corvus*	*albicollis*		White-necked Raven	*Leucocytozoon* sp.	L_WW6	KM056645
468630	2	Passeriformes	Corvidae	*Corvus*	*albicollis*		White-necked Raven	0	NA	NA
468625	2	Passeriformes	Corvidae	*Corvus*	*albicollis*		White-necked Raven	*Plasmodium* sp.	P_BUL07	KM056642
468615	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	Unknown	Coinfection	NA
468618	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	Unknown	Coinfection	NA
468617	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	*Haemoproteus lanii*	H_RBS4	KM056411
468616	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	0	NA	NA
468614	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	*Plasmodium* sp.	P_AFR10	KM056563
468614	1	Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	*Plasmodium* sp.	P_BUL07	KM056642
468355	1	Passeriformes	Emberizidae	*Emberiza*	*cabanisi*	*orientalis*	Cabanis's Bunting	*Parahaemoproteus* sp.	H_AFR90	KM056472
468354	1	Passeriformes	Emberizidae	*Emberiza*	*flaviventris*	*kalaharica*	African Golden-breasted Bunting	*Plasmodium* sp.	P_AFR47	KM056599
468354	1	Passeriformes	Emberizidae	*Emberiza*	*flaviventris*	*kalaharica*	kalaharica	*Plasmodium* sp.	P_AFR6	KM056605
468458	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	0	NA	NA
468482	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	0	NA	NA
468460	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	0	NA	NA
468461	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	0	NA	NA
468481	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	*Plasmodium* sp.	P_AFR40	KM056597
468459	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	*Plasmodium* sp.	P_AFR58	KM056604
468459	1	Passeriformes	Estrildidae	*Amandava*	*subflava*	*clarkei*	Zebra Waxbill	*Plasmodium* sp.	P_BUL07	KM056642
468427	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	Unknown	Coinfection	NA
468436	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Parahaemoproteus* sp.	H_AFR25	KM056444
468428	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Parahaemoproteus* sp.	H_AFR35	KM056447
468433	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR192	KM056510
468425	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR214	KM056531
468428	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR214	KM056531
468438	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR214	KM056531
468435	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR214	KM056531

468436	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR214	KM056531
468438	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR222	KM056536
468432	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR223	KM056537
468434	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Leucocytozoon* sp.	L_AFR223	KM056537
468431	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	0	NA	NA
468434	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Plasmodium* sp.	P_AFR132	KM056581
468432	2	Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	*Plasmodium* sp.	P_PSEGRI01	KM056637
468457	2	Passeriformes	Estrildidae	*Estrilda*	*astrild*	*cavendishi*	Common Waxbill	Unknown	Coinfection	NA
468455	2	Passeriformes	Estrildidae	*Estrilda*	*astrild*	*cavendishi*	Common Waxbill	0	NA	NA
468456	2	Passeriformes	Estrildidae	*Estrilda*	*astrild*	*cavendishi*	Common Waxbill	0	NA	NA
468475	1	Passeriformes	Estrildidae	*Estrilda*	*astrild*	*cavendishi*	Common Waxbill	*Plasmodium* sp.	P_BUL07	KM056642
468465	2	Passeriformes	Estrildidae	*Estrilda*	*melanotis*	*stuartirwini*	Yellow-bellied Waxbill	*Leucocytozoon* sp.	L_AFR177	KM056495
468463	2	Passeriformes	Estrildidae	*Estrilda*	*melanotis*	*stuartirwini*	Yellow-bellied Waxbill	*Leucocytozoon* sp.	L_AFR214	KM056531
468462	2	Passeriformes	Estrildidae	*Estrilda*	*melanotis*	*stuartirwini*	Yellow-bellied Waxbill	0	NA	NA
468464	2	Passeriformes	Estrildidae	*Estrilda*	*melanotis*	*stuartirwini*	Yellow-bellied Waxbill	*Plasmodium* sp.	P_AFR22	KM056590
468441	2	Passeriformes	Estrildidae	*Hypargos*	*niveoguttatus*	*macrospilotus*	Peters's Twinspot	*Parahaemoproteus* sp.	H_AFR59	KM056456
468439	2	Passeriformes	Estrildidae	*Hypargos*	*niveoguttatus*	*macrospilotus*	Peters's Twinspot	*Leucocytozoon* sp.	L_AFR212	KM056530
468440	2	Passeriformes	Estrildidae	*Hypargos*	*niveoguttatus*	*macrospilotus*	Peters's Twinspot	*Plasmodium* sp.	P_BUL07	KM056642
468442	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*	*haematocephala*	African Firefinch	*Leucocytozoon* sp.	L_AFR211	KM056529
468444	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*	*haematocephala*	African Firefinch	0	NA	NA
468443	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*	*haematocephala*	African Firefinch	*Plasmodium* sp.	P_RFF1	KM056632
479646	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*		African Firefinch	*Leucocytozoon* sp.	L_AFR157	KM056477
479646	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*		African Firefinch	*Leucocytozoon* sp.	L_AFR235	KM056549
479646	1	Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*		African Firefinch	*Plasmodium* sp.	P_AFR31	KM056594
468468	1	Passeriformes	Estrildidae	*Lonchura*	*cucullata*	*scutata*	Bronze Mannikin	*Parahaemoproteus* sp.	H_AFR25	KM056444
468472	1	Passeriformes	Estrildidae	*Lonchura*	*cucullata*	*scutata*	Bronze Mannikin	*Parahaemoproteus* sp.	H_AFR57	KM056455
468470	1	Passeriformes	Estrildidae	*Lonchura*	*cucullata*	*scutata*	Bronze Mannikin	0	NA	NA
468466	1	Passeriformes	Estrildidae	*Lonchura*	*cucullata*	*scutata*	Bronze Mannikin	*Plasmodium* sp.	P_BUL07	KM056642
468417	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Leucocytozoon* sp.	L_AFR214	KM056531
468414	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	0	NA	NA
468415	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	0	NA	NA
468420	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_AFR13	KM056579
468421	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_AFR91	KM056615
468421	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_AFR92	KM056616
468414	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_RFF1	KM056632
468417	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_RFF1	KM056632
468420	1	Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	*Plasmodium* sp.	P_RFF1	KM056632
468422	1	Passeriformes	Estrildidae	*Pytilia*	*melba*	*melba*	Green-winged Pytilia	*Plasmodium* sp.	P_AFR10	KM056563
468422	1	Passeriformes	Estrildidae	*Pytilia*	*melba*	*melba*	Green-winged Pytilia	*Plasmodium* sp.	P_COLL7	KM056625
468447	1	Passeriformes	Estrildidae	*Uraeginthus*	*angolensis*	*niassensis*	Southern Cordonbleu	0	NA	NA
468624	1	Passeriformes	Estrildidae	*Uraeginthus*	*angolensis*	*niassensis*	Southern Cordonbleu	*Plasmodium* sp.	P_GRW09	KM056631
468448	1	Passeriformes	Estrildidae	*Uraeginthus*	*angolensis*	*niassensis*	Southern Cordonbleu	*Plasmodium* sp.	P_RFF1	KM056632
467943	2	Passeriformes	Eurylaimidae	*Smithornis*	*capensis*	*albigularis*	African Broadbill	*Leucocytozoon* sp.	L_AFR175	KM056493
468382	2	Passeriformes	Fringillidae	*Serinus*	*canicollis*	*sassii*	Cape Canary	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468389	2	Passeriformes	Fringillidae	*Serinus*	*canicollis*	*sassii*	Cape Canary	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468383	2	Passeriformes	Fringillidae	*Serinus*	*canicollis*	*sassii*	Cape Canary	*Plasmodium* sp.	P_AFR101	KM056564
468356	2	Passeriformes	Fringillidae	*Serinus*	*citrinelloides*	*hypostictus*	African Citril	*Leucocytozoon* sp.	L_AFR244	KM056557
468376	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Parahaemoproteus* sp.	H_AFR51	KM056453
468378	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Parahaemoproteus* sp.	H_AFR63	KM056459
468374	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Parahaemoproteus* sp.	H_AFR72	KM056463
468374	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Parahaemoproteus* sp.	H_AFR73	KM056464
468375	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Parahaemoproteus* sp.	H_AFR75	KM056465
468375	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Leucocytozoon* sp.	L_AFR161	KM056480

468374	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Leucocytozoon* sp.	L_REB7	KM056647
468377	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Plasmodium* sp.	P_AFR83	KM056612
468377	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Plasmodium* sp.	P_GRW09	KM056631
468375	1	Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	*Plasmodium* sp.	P_WW3	NA
468358	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Parahaemoproteus* sp.	H_PYERY01	KM056418
468362	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Leucocytozoon* sp.	L_RECOB3	KM056648
468364	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Leucocytozoon* sp.	L_RECOB3	KM056648
468359	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468361	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468365	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	0	NA	NA
468366	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	0	NA	NA
468367	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	0	NA	NA
468370	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	0	NA	NA
468368	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Plasmodium* sp.	P_AFR28	KM056593
468362	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Plasmodium* sp.	P_AFR99	KM056620
468361	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Plasmodium* sp.	P_BUL07	KM056642
468369	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Plasmodium* sp.	P_MALNI02	KM056641
468359	2	Passeriformes	Fringillidae	*Serinus*	*striolatus*	*whytii*	Yellow-browed Seedeater	*Plasmodium* sp.	P_PSEGRI01	KM056637
467954	1	Passeriformes	Hirundinidae	*Delichon*	*urbica*	*urbica*	House Martin	*Leucocytozoon* sp.	L_AFR167	KM056486
467952	2	Passeriformes	Hirundinidae	*Hirundo*	*angolensis*	*angolensis*	Angola Swallow	*Parahaemoproteus* sp.	H_AFR103	KM056420
467953	2	Passeriformes	Hirundinidae	*Hirundo*	*angolensis*	*angolensis*	Angola Swallow	*Plasmodium* sp.	P_BUL07	KM056642
467949	2	Passeriformes	Hirundinidae	*Psalidoprocne*	*albiceps*	*albiceps*	White-headed Sawwing	*Plasmodium* sp.	P_GRW09	KM056631
467950	2	Passeriformes	Hirundinidae	*Psalidoprocne*	*albiceps*	*albiceps*	White-headed Sawwing	*Plasmodium* sp.	P_GRW09	KM056631
468040	1	Passeriformes	Laniidae	*Lanius*	*collaris*	*capelli*	Common Fiscal	Unknown	Coinfection	NA
468038	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468030	2	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Leucocytozoon* sp.	L_AFR203	KM056521
468027	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Leucocytozoon* sp.	L_AFR218	KM056533
468030	2	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Leucocytozoon* sp.	L_AFR233	KM056547
468030	2	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR6	KM056605
468026	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR6	KM056605
468037	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR6	KM056605
468029	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR6	KM056605
468027	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR9	KM056614
468037	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_AFR9	KM056614
468038	1	Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	*Plasmodium* sp.	P_BUL07	KM056642
468031	2	Passeriformes	Malaconotidae	*Laniarius*	*fulleborni*	*fulleborni*	Fuelleborn's Boubou	*Leucocytozoon* sp.	L_AFR219	KM056534
468033	2	Passeriformes	Malaconotidae	*Laniarius*	*fulleborni*	*fulleborni*	Fuelleborn's Boubou	*Leucocytozoon* sp.	L_AFR219	KM056534
468032	2	Passeriformes	Malaconotidae	*Laniarius*	*fulleborni*	*fulleborni*	Fuelleborn's Boubou	*Leucocytozoon* sp.	L_AFR242	KM056555
468031	2	Passeriformes	Malaconotidae	*Laniarius*	*fulleborni*	*fulleborni*	Fuelleborn's Boubou	*Plasmodium* sp.	P_AFR6	KM056605
468032	2	Passeriformes	Malaconotidae	*Laniarius*	*fulleborni*	*fulleborni*	Fuelleborn's Boubou	*Plasmodium* sp.	P_BUL07	KM056642
468036	1	Passeriformes	Malaconotidae	*Malaconotus*	*blanchoti*	*hypopyrrhus*	Grey-headed Bush-Shrike	*Plasmodium* sp.	P_AFR22	KM056590
468025	2	Passeriformes	Malaconotidae	*Tchagra*	*australis*	*congener*	Brown-crowned Tchagra	*Leucocytozoon* sp.	L_AFR222	KM056536
468025	2	Passeriformes	Malaconotidae	*Tchagra*	*australis*	*congener*	Brown-crowned Tchagra	*Plasmodium* sp.	P_AFR145	KM056586
468025	2	Passeriformes	Malaconotidae	*Tchagra*	*australis*	*congener*	Brown-crowned Tchagra	*Plasmodium* sp.	P_AFR146	KM056587
468021	1	Passeriformes	Malaconotidae	*Tchagra*	*minuta*	*anchietae*	Anchieta's Tchagra	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468022	1	Passeriformes	Malaconotidae	*Tchagra*	*senegala*	*armena*	Black-crowned Tchagra	0	NA	NA
468023	1	Passeriformes	Malaconotidae	*Tchagra*	*senegala*	*armena*	Black-crowned Tchagra	0	NA	NA
468289	1	Passeriformes	Monarchidae	*Terpsiphone*	*viridis*	*plumbeiceps*	African Paradise-flycatcher	*Parahaemoproteus* sp.	H_TERUF01	KM056415
468293	2	Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	*Leucocytozoon* sp.	L_AFR183	KM056501
468294	2	Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	*Leucocytozoon* sp.	L_AFR234	KM056548
468292	2	Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	*Leucocytozoon* sp.	L_WW6	KM056645
468296	2	Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	*Plasmodium* sp.	P_AFR140	KM056584
468296	2	Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	*Plasmodium* sp.	P_BUL07	KM056642

467960	2	Passeriformes	Motacillidae	*Anthus*	*novaeseelandiae*	*lichenya*	African Pipit	0	NA	NA
467959	2	Passeriformes	Motacillidae	*Anthus*	*novaeseelandiae*	*lichenya*	African Pipit	*Plasmodium* sp.	P_PBPIP1	KM056639
468102	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468097	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Parahaemoproteus* sp.	H_AFR121	KM056427
468098	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Parahaemoproteus* sp.	H_AFR122	KM056428
468103	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_AFR186	KM056504
468107	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_AFR222	KM056536
468100	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_AFR226	KM056540
468097	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_AFR239	KM056553
468098	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_AFR239	KM056553
468101	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_WW6	KM056645
468106	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_WW6	KM056645
468106	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468105	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	0	NA	NA
468103	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Plasmodium* sp.	P_BUL07	KM056642
468107	2	Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	*Plasmodium* sp.	P_MALNI02	KM056641
468091	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_AFR215	KM056532
468094	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_AFR222	KM056536
468086	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_AFR225	KM056539
468096	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_AFR229	KM056543
468086	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_RECOB3	KM056648
468092	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_RECOB3	KM056648
468089	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Leucocytozoon* sp.	L_ZOABY02	KM056650
468093	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	0	NA	NA
468095	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	0	NA	NA
468091	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Plasmodium* sp.	P_AFR108	KM056569
468085	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Plasmodium* sp.	P_AFR93	KM056617
468085	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Plasmodium* sp.	P_AFR94	KM056618
468090	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Plasmodium* sp.	P_BT8	KM056624
468096	2	Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	*Plasmodium* sp.	P_BUL07	KM056642
468081	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Parahaemoproteus* sp.	H_AFR144	KM056436
468083	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Parahaemoproteus* sp.	H_AFR59	KM056456
468082	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Parahaemoproteus* sp.	H_QUERY01	KM056416
468083	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Leucocytozoon* sp.	L_AFR205	KM056523
468084	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Leucocytozoon* sp.	L_AFR212	KM056530
468080	1	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Leucocytozoon* sp.	L_AFR218	KM056533
468078	1	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	0	NA	NA
468083	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Plasmodium* sp.	P_AFR147	KM056588
468080	1	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Plasmodium* sp.	P_COLL11	KM056636
468084	2	Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	*Plasmodium* sp.	P_GRW09	KM056631
468131	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Parahaemoproteus* sp.	H_AFR122	KM056428
468133	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Parahaemoproteus* sp.	H_AFR122	KM056428
468138	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Parahaemoproteus* sp.	H_AFR139	KM056434
468133	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Leucocytozoon* sp.	L_AFR211	KM056529
468135	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Leucocytozoon* sp.	L_AFR220	KM056535
468134	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Leucocytozoon* sp.	L_AFR229	KM056543
468132	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Plasmodium* sp.	P_BUL07	KM056642
468135	2	Passeriformes	Muscicapidae	*Erithacus*	*sharpei*	*sharpei*	Sharpe's Akalat	*Plasmodium* sp.	P_BUL07	KM056642
468044	1	Passeriformes	Muscicapidae	*Erythropygia*	*barbata*		Miombo Scrub-Robin	*Plasmodium* sp.	P_AFR65	KM056607
468043	1	Passeriformes	Muscicapidae	*Erythropygia*	*barbata*		Miombo Scrub-Robin	*Plasmodium* sp.	P_COLL11	KM056636
468045	1	Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	*Leucocytozoon* sp.	L_AFR154	KM056474
468045	1	Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	*Plasmodium* sp.	P_COLL11	KM056636
468046	1	Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	*Plasmodium* sp.	P_COLL11	KM056636

468047	1	Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	*Plasmodium* sp.	P_COLL11	KM056636
468048	1	Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	*Plasmodium* sp.	P_PSEGRI01	KM056637
468309	2	Passeriformes	Muscicapidae	*Ficedula*	*albicollis*	*albicollis*	Collared Flycatcher	*Haemoproteus pallidus*	H_COLL2	KM056413
468301	2	Passeriformes	Muscicapidae	*Muscicapa*	*adusta*	*subadusta*	African Dusky Flycatcher	Unknown	Coinfection	NA
468302	2	Passeriformes	Muscicapidae	*Muscicapa*	*adusta*	*subadusta*	African Dusky Flycatcher	*Parahaemoproteus* sp.	H_AFR150	KM056439
468307	2	Passeriformes	Muscicapidae	*Muscicapa*	*coerulescens*	*impavida*	Ashy Flycatcher	*Parahaemoproteus* sp.	H_AFR148	KM056437
468111	1	Passeriformes	Muscicapidae	*Myrmecocichla*	*arnotti*	*arnotti*	White-headed Black-Chat	*Plasmodium* sp.	P_AFR23	KM056591
468113	1	Passeriformes	Muscicapidae	*Myrmecocichla*	*arnotti*	*arnotti*	White-headed Black-Chat	*Plasmodium* sp.	P_AFR68	KM056608
468111	1	Passeriformes	Muscicapidae	*Myrmecocichla*	*arnotti*	*arnotti*	White-headed Black-Chat	*Plasmodium* sp.	P_BT8	KM056624
468113	1	Passeriformes	Muscicapidae	*Myrmecocichla*	*arnotti*	*arnotti*	White-headed Black-Chat	*Plasmodium* sp.	P_RFF1	KM056632
468066	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Parahaemoproteus* sp.	H_AFR126	KM056429
468059	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Parahaemoproteus* sp.	H_AFR153	KM056441
468060	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Parahaemoproteus* sp.	H_AFR25	KM056444
468068	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Leucocytozoon* sp.	L_AFR196	KM056514
468063	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Leucocytozoon* sp.	L_AFR211	KM056529
468070	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Leucocytozoon* sp.	L_AFR237	KM056551
468062	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Leucocytozoon* sp.	L_RECOB3	KM056648
468056	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	0	NA	NA
468057	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	0	NA	NA
468061	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	0	NA	NA
468067	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	0	NA	NA
468062	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_AFR124	KM056575
468064	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_AFR125	KM056576
468069	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_AFR134	KM056582
468062	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_BUL07	KM056642
468058	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_GRW09	KM056631
468065	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_GRW09	KM056631
468055	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_MALNI02	KM056641
468050	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_PSEGRI01	KM056637
468062	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_PSEGRI01	KM056637
468064	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_PSEGRI01	KM056637
468070	2	Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	*Plasmodium* sp.	P_PSEGRI01	KM056637
468115	1	Passeriformes	Muscicapidae	*Saxicola*	*torquata*	*promiscua*	Common Stonechat	0	NA	NA
468116	2	Passeriformes	Muscicapidae	*Saxicola*	*torquata*	*promiscua*	Common Stonechat	0	NA	NA
468339	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	*Parahaemoproteus* sp.	H_AFR111	KM056422
468344	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	*Parahaemoproteus* sp.	H_AFR116	KM056424
468341	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	*Parahaemoproteus* sp.	H_AFR8	KM056468
468342	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	*Leucocytozoon* sp.	L_AFR214	KM056531
468343	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	0	NA	NA
468345	2	Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	0	NA	NA
468351	1	Passeriformes	Nectariniidae	*Nectarinia*	*amethystina*	*kirkii*	Amethyst Sunbird	*Parahaemoproteus* sp.	H_AFR85	KM056471
468351	1	Passeriformes	Nectariniidae	*Nectarinia*	*amethystina*	*kirkii*	Amethyst Sunbird	*Leucocytozoon* sp.	L_AFR164	KM056483
468324	2	Passeriformes	Nectariniidae	*Nectarinia*	*famosa*	*aeneigularis*	Malachite Sunbird	0	NA	NA
468326	2	Passeriformes	Nectariniidae	*Nectarinia*	*famosa*	*aeneigularis*	Malachite Sunbird	0	NA	NA
468352	2	Passeriformes	Nectariniidae	*Nectarinia*	*kilimensis*	*arturi*	Bronze Sunbird	*Parahaemoproteus* sp.	H_AFR116	KM056424
468333	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Parahaemoproteus* sp.	H_AFR116	KM056424
468334	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468335	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Leucocytozoon* sp.	L_AFR184	KM056502
468334	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Leucocytozoon* sp.	L_AFR219	KM056534
468338	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Leucocytozoon* sp.	L_AFR246	KM056559
468331	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	0	NA	NA
468332	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	0	NA	NA
468335	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_AFR127	KM056577

468337	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_AFR129	KM056578
468336	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_AFR152	KM056589
468335	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_AFR6	KM056605
468336	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_PSEGRI01	KM056637
468330	2	Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	*Plasmodium* sp.	P_PSEGRI01	KM056637
468318	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	Unknown	Coinfection	NA
468318	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Haemoproteus cyanomitrae*	H_CYAOLI05	KM056417
468319	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Haemoproteus cyanomitrae*	H_CYAOLI05	KM056417
468320	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Haemoproteus cyanomitrae*	H_CYAOLI05	KM056417
468321	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Haemoproteus cyanomitrae*	H_CYAOLI05	KM056417
468323	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Haemoproteus cyanomitrae*	H_CYAOLI05	KM056417
468323	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Leucocytozoon* sp.	L_AFR204	KM056522
468323	2	Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	*Leucocytozoon* sp.	L_ANLAT16	KM056643
468350	1	Passeriformes	Nectariniidae	*Nectarinia*	*senegalensis*	*gutturalis*	Scarlet-chested Sunbird	*Parahaemoproteus* sp.	H_AFR78	KM056467
468348	1	Passeriformes	Nectariniidae	*Nectarinia*	*senegalensis*	*gutturalis*	Scarlet-chested Sunbird	*Plasmodium* sp.	P_CYAOLI04	KM056640
468346	1	Passeriformes	Nectariniidae	*Nectarinia*	*venusta*	*falkensteini*	Variable Sunbird	*Parahaemoproteus* sp.	H_AFR62	KM056458
468347	1	Passeriformes	Nectariniidae	*Nectarinia*	*venusta*	*falkensteini*	Variable Sunbird	*Plasmodium* sp.	P_AFR10	KM056563
468347	1	Passeriformes	Nectariniidae	*Nectarinia*	*venusta*	*falkensteini*	Variable Sunbird	*Plasmodium* sp.	P_COLL7	KM056625
468613	1	Passeriformes	Oriolidae	*Oriolus*	*auratus*	*notatus*	African Golden-Oriole	Unknown	Coinfection	NA
468609	1	Passeriformes	Oriolidae	*Oriolus*	*auratus*	*notatus*	African Golden-Oriole	*Parahaemoproteus* sp.	H_AFR53	KM056454
468315	2	Passeriformes	Paridae	*Parus*	*griseiventris*		Miombo Tit	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468315	2	Passeriformes	Paridae	*Parus*	*griseiventris*		Miombo Tit	*Plasmodium* sp.	P_AFR110	KM056570
468312	1	Passeriformes	Paridae	*Parus*	*niger*	*niger*	Southern Black Tit	*Leucocytozoon* sp.	L_AFR159	KM056479
468312	1	Passeriformes	Paridae	*Parus*	*niger*	*niger*	Southern Black Tit	*Plasmodium* sp.	P_AFR60	KM056606
468314	1	Passeriformes	Passeridae	*Parus*	*rufiventris*		Rufous-bellied Tit	*Leucocytozoon* sp.	L_AFR163	KM056482
468486	1	Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	*Parahaemoproteus* sp.	H_AFR29	KM056445
468488	1	Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	*Parahaemoproteus* sp.	H_AFR42	KM056449
468493	1	Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	*Plasmodium* sp.	P_AFR10	KM056563
468493	1	Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	*Plasmodium* sp.	P_BUL07	KM056642
468491	1	Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	*Plasmodium* sp.	P_GRW09	KM056631
468495	1	Passeriformes	Passeridae	*Plocepasser*	*rufoscapulatus*		Chestnut-backed Sparrow-Weaver	0	NA	NA
468496	1	Passeriformes	Passeridae	*Plocepasser*	*rufoscapulatus*		Chestnut-backed Sparrow-Weaver	*Plasmodium* sp.	P_PSEGRI01	KM056637
468273	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468274	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468276	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468288	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468278	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	Unknown	Coinfection	NA
468287	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Parahaemoproteus* sp.	H_AFR149	KM056438
468286	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Parahaemoproteus* sp.	H_AFR59	KM056456
468278	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Parahaemoproteus* sp.	H_AFR8	KM056468
468279	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR180	KM056498
468282	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR190	KM056508
468283	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR193	KM056511
468287	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR206	KM056524
468266	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR211	KM056529
468279	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR224	KM056538
468280	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR224	KM056538
468281	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR224	KM056538
468282	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR229	KM056543
468286	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Leucocytozoon* sp.	L_AFR247	KM056560
468267	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	0	NA	NA
468272	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	0	NA	NA
468275	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	0	NA	NA

468277	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_AFR117	KM056572
468281	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_AFR131	KM056580
468286	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_AFR135	KM056583
468283	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_BUL07	KM056642
468268	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_COLL11	KM056636
468274	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_MALNI02	KM056641
468276	2	Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	*Plasmodium* sp.	P_PSEGRI01	KM056637
468265	1	Passeriformes	Platysteiridae	*Batis*	*molitor*	*palliditergum*	Chinspot Batis	0	NA	NA
468264	1	Passeriformes	Platysteiridae	*Batis*	*molitor*	*palliditergum*	Chinspot Batis	*Plasmodium* sp.	P_AFR28	KM056593
468558	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Leucocytozoon* sp.	L_AFR225	KM056539
468557	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	0	NA	NA
468563	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Plasmodium* sp.	P_AFR86	KM056613
468559	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Plasmodium* sp.	P_COLL7	KM056625
468560	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Plasmodium* sp.	P_COLL7	KM056625
468558	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Plasmodium* sp.	P_GRW10	KM056622
468559	1	Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	*Plasmodium* sp.	P_RFF1	KM056632
468554	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Parahaemoproteus* sp.	H_AFR19	KM056442
468554	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Leucocytozoon* sp.	L_AFR156	KM056476
468554	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Leucocytozoon* sp.	L_REB6	KM056644
468548	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Leucocytozoon* sp.	L_SATEC01	NA
468547	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	0	NA	NA
468553	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Plasmodium* sp.	P_MALNI02	KM056641
468548	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Plasmodium* sp.	P_RFF1	KM056632
468552	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Plasmodium* sp.	P_WW3	NA
468552	1	Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	*Plasmodium* sp.	P_WW4	KM056626
468546	1	Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	Unknown	Coinfection	NA
468542	1	Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	*Leucocytozoon* sp.	L_AFR218	KM056533
468542	1	Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	*Plasmodium* sp.	P_AFR10	KM056563
468545	1	Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	*Plasmodium* sp.	P_GRW10	KM056622
468538	1	Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	*Plasmodium* sp.	P_RFF1	KM056632
468533	2	Passeriformes	Ploceidae	*Euplectes*	*hartlaubi*	*psammocromius*	Marsh Widowbird	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468535	2	Passeriformes	Ploceidae	*Euplectes*	*hartlaubi*	*psammocromius*	Marsh Widowbird	*Leucocytozoon* sp.	L_AFR169	KM056488
468535	2	Passeriformes	Ploceidae	*Euplectes*	*hartlaubi*	*psammocromius*	Marsh Widowbird	*Leucocytozoon* sp.	L_AFR214	KM056531
468536	2	Passeriformes	Ploceidae	*Euplectes*	*hartlaubi*	*psammocromius*	Marsh Widowbird	0	NA	NA
468533	2	Passeriformes	Ploceidae	*Euplectes*	*hartlaubi*	*psammocromius*	Marsh Widowbird	*Plasmodium* sp.	P_BUL07	KM056642
468505	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468497	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_AFR168	KM056487
468498	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_AFR170	KM056489
468502	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_AFR172	KM056490
468498	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_AFR228	KM056542
468500	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_RECOB3	KM056648
468499	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_SATEC01	NA
468502	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_SATEC01	NA
468503	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Leucocytozoon* sp.	L_YMWD2	KM056649
468506	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	0	NA	NA
468501	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Plasmodium* sp.	P_AFR28	KM056593
468504	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Plasmodium* sp.	P_AFR6	KM056605
468505	2	Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	*Plasmodium* sp.	P_LINOLI01	KM056629
468522	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Leucocytozoon* sp.	L_AFR218	KM056533
468523	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_AFR10	KM056563
468521	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_AFR49	KM056600
468520	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468521	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_MALNI02	KM056641

468522	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468576	1	Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	*Plasmodium* sp.	P_WW3	NA
468529	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468526	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Leucocytozoon* sp.	L_AFR211	KM056529
468526	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium* sp.	P_AFR10	KM056563
468525	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium* sp.	P_BT8	KM056624
468527	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium* sp.	P_BT8	KM056624
468526	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium relictum*	P_LZFUS01	KM056627
468528	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468529	1	Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468507	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Leucocytozoon* sp.	L_AFR155	KM056475
468509	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Leucocytozoon* sp.	L_AFR219	KM056534
468517	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	0	NA	NA
468514	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_BT8	KM056624
468507	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468508	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468509	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468510	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468511	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468513	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468514	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468515	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468516	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468518	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium* sp.	P_MALNI02	KM056641
468512	1	Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	*Plasmodium megalogluobularis*	P_PYSUN1	KM056628
468582	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	Unknown	Coinfection	NA
468583	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	Unknown	Coinfection	NA
468580	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Parahaemoproteus* sp.	H_AFR29	KM056445
468581	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Parahaemoproteus* sp.	H_QUERY01	KM056416
468585	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Parahaemoproteus* sp.	H_RBQ11	KM056419
468585	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Leucocytozoon* sp.	L_AFR166	KM056485
468577	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Plasmodium* sp.	P_AFR10	KM056563
468579	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Plasmodium* sp.	P_AFR10	KM056563
468584	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Plasmodium* sp.	P_GRW09	KM056631
468577	1	Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	*Plasmodium* sp.	P_PLOVEL01	KM056630
468004	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	*Parahaemoproteus* sp.	H_AFR141	KM056435
468005	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	*Parahaemoproteus* sp.	H_AFR141	KM056435
468006	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	*Parahaemoproteus* sp.	H_AFR141	KM056435
468008	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	*Parahaemoproteus* sp.	H_QUERY01	KM056416
468008	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	*Leucocytozoon* sp.	L_AFR237	KM056551
468007	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	0	NA	NA
468012	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468013	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468011	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon* sp.	L_AFR226	KM056540
468015	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon* sp.	L_AFR226	KM056540
468010	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon* sp.	L_AFR227	KM056541
468009	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Leucocytozoon* sp.	L_AFR234	KM056548
468010	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Plasmodium* sp.	P_AFR123	KM056574
468009	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Plasmodium* sp.	P_BUL07	KM056642
468010	2	Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Sharpe's Yellow-streaked Greenbul	*Plasmodium* sp.	P_BUL07	KM056642
467999	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468001	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Haemoproteus sanguinus*	H_BUL2	KM056412
467995	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Haemoproteus belopolskyi*	H_MW1	KM056408

467994	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Haemoproteus belopolskyi*	H_SW1	KM056409
467993	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon* sp.	L_AFR158	KM056478
468001	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon* sp.	L_AFR165	KM056484
468002	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon* sp.	L_AFR174	KM056492
468003	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon* sp.	L_AFR225	KM056539
467995	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Leucocytozoon* sp.	L_AFR238	KM056552
467991	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	0	NA	NA
468000	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Plasmodium* sp.	P_BUL07	KM056642
468003	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Plasmodium* sp.	P_BUL07	KM056642
467999	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Plasmodium* sp.	P_GRW10	KM056622
467996	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Plasmodium relictum*	P_LZFUS01	KM056627
467994	1	Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	*Plasmodium* sp.	P_PSEGRI01	KM056637
467966	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
467964	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Leucocytozoon* sp.	L_AFR194	KM056512
467965	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Leucocytozoon* sp.	L_AFR220	KM056535
467965	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Leucocytozoon* sp.	L_AFR230	KM056544
467964	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Plasmodium* sp.	P_GRW09	KM056631
467966	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	*Plasmodium* sp.	P_PSEGRI01	KM056637
467973	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
467977	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
467969	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	Unknown	Coinfection	NA
467969	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR103	KM056420
467971	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR103	KM056420
467974	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR103	KM056420
467978	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR103	KM056420
467982	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR103	KM056420
467980	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR130	KM056430
467983	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR130	KM056430
467981	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Parahaemoproteus* sp.	H_AFR8	KM056468
467980	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR188	KM056506
467982	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR189	KM056507
467983	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR191	KM056509
467986	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR195	KM056513
467990	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR200	KM056518
467976	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR212	KM056530
467978	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR212	KM056530
467985	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR215	KM056532
467967	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR223	KM056537
467970	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR223	KM056537
467981	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR231	KM056545
467987	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR231	KM056545
467971	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR236	KM056550
467974	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR236	KM056550
467988	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR236	KM056550
467989	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR236	KM056550
467981	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Leucocytozoon* sp.	L_AFR239	KM056553
467972	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	0	NA	NA
467975	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	0	NA	NA
467986	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Plasmodium* sp.	P_AFR65	KM056607
467990	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Plasmodium* sp.	P_BUL07	KM056642
467967	2	Passeriformes	Pycnonotidae	*Pycnonotus*	*tephrolaemus*	*fusciceps*	Southern Mountain Greenbul	*Plasmodium* sp.	P_COLL7	KM056625
468316	1	Passeriformes	Remizidae	*Anthoscopus*	*caroli*	*robertsi*	African Penduline-Tit	0	NA	NA
468291	2	Passeriformes	Stenostiridae	*Elminia*	*albonotata*		White-tailed Crested-Flycatcher	0	NA	NA

468602	1	Passeriformes	Sturnidae	*Lamprotornis*	*chalybaeus*	*sycobius*	Greater Blue-eared Starling	Unknown	Coinfection	NA
468601	1	Passeriformes	Sturnidae	*Lamprotornis*	*chalybaeus*	*sycobius*	Greater Blue-eared Starling	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468601	1	Passeriformes	Sturnidae	*Lamprotornis*	*chalybaeus*	*sycobius*	Greater Blue-eared Starling	*Leucocytozoon* sp.	L_AFR211	KM056529
468601	1	Passeriformes	Sturnidae	*Lamprotornis*	*chalybaeus*	*sycobius*	Greater Blue-eared Starling	*Plasmodium* sp.	P_BUL07	KM056642
468603	1	Passeriformes	Sturnidae	*Lamprotornis*	*chloropterus*	*elisabeth*	Lesser Blue-eared Glossy-Starling	*Parahaemoproteus* sp.	H_AFR41	KM056448
468607	1	Passeriformes	Sturnidae	*Lamprotornis*	*chloropterus*	*elisabeth*	Lesser Blue-eared Glossy-Starling	*Parahaemoproteus* sp.	H_AFR76	KM056466
468605	1	Passeriformes	Sturnidae	*Lamprotornis*	*chloropterus*	*elisabeth*	Lesser Blue-eared Glossy-Starling	0	NA	NA
468608	1	Passeriformes	Sturnidae	*Lamprotornis*	*chloropterus*	*elisabeth*	Lesser Blue-eared Glossy-Starling	0	NA	NA
468590	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Parahaemoproteus* sp.	H_AFR8	KM056468
468595	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Parahaemoproteus* sp.	H_AFR81	KM056469
468600	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Parahaemoproteus* sp.	H_AFR84	KM056470
468594	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Leucocytozoon* sp.	L_AFR211	KM056529
468593	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Leucocytozoon* sp.	L_AFR228	KM056542
468594	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Plasmodium* sp.	P_ACCTAC01	KM056621
468593	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Plasmodium* sp.	P_AFR80	KM056610
468595	1	Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	*Plasmodium* sp.	P_AFR82	KM056611
468589	2	Passeriformes	Sturnidae	*Onychognathus*	*tenuirostris*	*theresae*	Slender-billed Starling	*Parahaemoproteus* sp.	H_AFR137	KM056432
468589	2	Passeriformes	Sturnidae	*Onychognathus*	*tenuirostris*	*theresae*	Slender-billed Starling	*Leucocytozoon* sp.	L_AFR215	KM056532
468172	1	Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	*Haemoproteus belopolskyi*	H_ARW1	KM056407
468171	1	Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	*Haemoproteus belopolskyi*	H_MW1	KM056408
468170	1	Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	*Haemoproteus payevski*	H_RW1	KM056406
468169	1	Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	0	NA	NA
468173	1	Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	0	NA	NA
468168	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	*Parahaemoproteus* sp.	H_AFR85	KM056471
468162	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	*Haemoproteus pallidus*	H_COLL2	KM056413
468164	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	0	NA	NA
468162	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	*Plasmodium* sp.	P_BUL07	KM056642
468168	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	*Plasmodium* sp.	P_BUL07	KM056642
468167	1	Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	*Plasmodium* sp.	P_GRW09	KM056631
468150	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	Unknown	Coinfection	NA
468156	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Parahaemoproteus* sp.	H_AFR116	KM056424
468157	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Haemoproteus micronuclearis*	H_QUERY01	KM056416
468155	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Leucocytozoon* sp.	L_AFR176	KM056494
468149	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	0	NA	NA
468147	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	0	NA	NA
468153	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	0	NA	NA
468154	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	0	NA	NA
468155	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Plasmodium* sp.	P_AFR115	KM056571
468152	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Plasmodium* sp.	P_MALNI02	KM056641
468155	2	Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	*Plasmodium* sp.	P_MALNI02	KM056641
468247	2	Passeriformes	Sylviidae	*Chloropeta*	*natalensis*	*massaica*	Dark-capped Yellow Warbler	*Leucocytozoon* sp.	L_AFR241	KM056554
468248	2	Passeriformes	Sylviidae	*Chloropeta*	*natalensis*	*massaica*	Dark-capped Yellow Warbler	0	NA	NA
468247	2	Passeriformes	Sylviidae	*Chloropeta*	*natalensis*	*massaica*	Dark-capped Yellow Warbler	*Plasmodium* sp.	P_AFR104	KM056565
468240	2	Passeriformes	Sylviidae	*Chloropeta*	*similis*		Mountain Yellow Warbler	*Leucocytozoon* sp.	L_RECOB3	KM056648
468239	2	Passeriformes	Sylviidae	*Chloropeta*	*similis*		Mountain Yellow Warbler	*Leucocytozoon* sp.	L_WW6	KM056645
468243	2	Passeriformes	Sylviidae	*Chloropeta*	*similis*		Mountain Yellow Warbler	0	NA	NA
468245	2	Passeriformes	Sylviidae	*Chloropeta*	*similis*		Mountain Yellow Warbler	0	NA	NA
468244	2	Passeriformes	Sylviidae	*Chloropeta*	*similis*		Mountain Yellow Warbler	*Plasmodium* sp.	P_SYBOR11	KM056638
468179	2	Passeriformes	Sylviidae	*Phylloscopus*	*ruficapilla*	*johnstoni*	Yellow-throated Woodland-Warbler	*Leucocytozoon* sp.	L_AFR198	KM056516
468179	2	Passeriformes	Sylviidae	*Phylloscopus*	*ruficapilla*	*johnstoni*	Yellow-throated Woodland-Warbler	*Leucocytozoon* sp.	L_AFR199	KM056517
468178	2	Passeriformes	Sylviidae	*Phylloscopus*	*ruficapilla*	*johnstoni*	Yellow-throated Woodland-Warbler	0	NA	NA
468177	2	Passeriformes	Sylviidae	*Phylloscopus*	*ruficapilla*	*johnstoni*	Yellow-throated Woodland-Warbler	*Plasmodium* sp.	P_BUL07	KM056642
468183	1	Passeriformes	Sylviidae	*Phylloscopus*	*trochilus*		Willow Warbler	*Parahaemoproteus* sp.	H_WW1	KM056414

468184	1	Passeriformes	Sylviidae	*Phylloscopus*	*trochilus*		Willow Warbler	0	NA	NA
468187	2	Passeriformes	Sylviidae	*Sylvia*	*atricapilla*	*atricapilla*	Eurasian Blackcap	0	NA	NA
468185	1	Passeriformes	Sylviidae	*Sylvia*	*borin*		Garden Warbler	Unknown	Coinfection	NA
468186	2	Passeriformes	Sylviidae	*Sylvia*	*borin*		Garden Warbler	*Haemoproteus parabelopolskyi*	H_SYBOR01	KM056410
468186	2	Passeriformes	Sylviidae	*Sylvia*	*borin*		Garden Warbler	*Leucocytozoon* sp.	L_AFR211	KM056529
468143	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468141	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon* sp.	L_AFR185	KM056503
468142	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon* sp.	L_AFR212	KM056530
468140	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon* sp.	L_AFR215	KM056532
468142	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon* sp.	L_AFR215	KM056532
468139	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Leucocytozoon* sp.	L_AFR243	KM056556
468140	2	Passeriformes	Timaliidae	*Alcippe*	*abyssinica*	*stierlingi*	African Hill Babbler	*Plasmodium* sp.	P_BUL07	KM056642
468144	2	Passeriformes	Timaliidae	*Trichastoma*	*pyrrhopterum*		Mountain Illadopsis	Unknown	Coinfection	NA
468144	2	Passeriformes	Timaliidae	*Trichastoma*	*pyrrhopterum*		Mountain Illadopsis	*Parahaemoproteus* sp.	H_AFR71	KM056462
468072	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Parahaemoproteus* sp.	H_AFR103	KM056420
468075	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Leucocytozoon* sp.	L_AFR179	KM056497
468074	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Leucocytozoon* sp.	L_AFR220	KM056535
468075	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Leucocytozoon* sp.	L_AFR220	KM056535
468073	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Leucocytozoon* sp.	L_AFR226	KM056540
468073	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Leucocytozoon* sp.	L_AFR232	KM056546
468071	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	0	NA	NA
468075	2	Passeriformes	Turdidae	*Alethe*	*fuelleborni*	*fuelleborni*	White-chested Alethe (nominate)	*Plasmodium* sp.	P_PSEGRI01	KM056637
468127	2	Passeriformes	Turdidae	*Turdus*	*abyssinicus*	*nyikae*	Olive Thrush	*Parahaemoproteus* sp.	H_AFR103	KM056420
468127	2	Passeriformes	Turdidae	*Turdus*	*abyssinicus*	*nyikae*	Olive Thrush	*Leucocytozoon* sp.	L_AFR236	KM056550
468130	2	Passeriformes	Turdidae	*Turdus*	*abyssinicus*	*nyikae*	Olive Thrush	*Leucocytozoon* sp.	L_AFR248	KM056561
468128	2	Passeriformes	Turdidae	*Turdus*	*abyssinicus*	*nyikae*	Olive Thrush	*Plasmodium* sp.	P_RFF1	KM056632
468122	1	Passeriformes	Turdidae	*Turdus*	*libonyanus*	*tropicalis*	Kurrichane Thrush	0	NA	NA
468123	1	Passeriformes	Turdidae	*Turdus*	*libonyanus*	*tropicalis*	Kurrichane Thrush	*Plasmodium* sp.	P_AFTRU4	KM056635
468126	1	Passeriformes	Turdidae	*Turdus*	*libonyanus*	*tropicalis*	Kurrichane Thrush	*Plasmodium* sp.	P_AFTRU4	KM056635
468118	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	*Leucocytozoon spp.*	Coinfection	*Leucocytozoon* spp.
468119	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	*Parahaemoproteus* sp.	H_AFR130	KM056430
468119	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	*Leucocytozoon* sp.	L_AFR187	KM056505
468121	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	*Leucocytozoon* sp.	L_AFR201	KM056519
468120	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	0	NA	NA
468121	2	Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	*Plasmodium elongatum*	P_GRW06	KM056633
468484	1	Passeriformes	Viduidae	*Vidua*	*macroura*		Pin-tailed Whydah	0	NA	NA
468398	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	Unknown	Coinfection	NA
468394	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_AFR96	KM056473
468395	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_AFR96	KM056473
468399	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_AFR96	KM056473
468400	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_AFR96	KM056473
468396	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_YEWE2	KM056405
468401	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_YEWE2	KM056405
468402	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_YEWE2	KM056405
468398	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468393	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468397	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468405	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468406	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468409	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Parahaemoproteus* sp.	H_ZOSMAD01	KM056404
468408	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR197	KM056515
468395	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529
468397	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529

468399	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529
468400	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529
468401	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529
468402	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR211	KM056529
468405	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR212	KM056530
468406	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR212	KM056530
468409	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Leucocytozoon* sp.	L_AFR212	KM056530
468396	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Plasmodium* sp.	P_AFR97	KM056619
468398	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Plasmodium* sp.	P_BUL07	KM056642
468402	2	Passeriformes	Zosteropidae	*Zosterops*	*senegalensis*	*stierlingi*	Yellow White-eye	*Plasmodium* sp.	P_MALNI02	KM056641
467924	1	Piciformes	Indicatoridae	*Indicator*	*indicator*		Greater Honeyguide	0	NA	NA
467926	1	Piciformes	Indicatoridae	*Indicator*	*indicator*		Greater Honeyguide	*Plasmodium* sp.	P_AFR54	KM056602
467926	1	Piciformes	Indicatoridae	*Indicator*	*indicator*		Greater Honeyguide	*Plasmodium* sp.	P_AFR55	KM056603
467922	1	Piciformes	Indicatoridae	*Indicator*	*minor*	*teitensis*	Lesser Honeyguide	0	NA	NA
467928	2	Piciformes	Indicatoridae	*Indicator*	*variegatus*	*variegatus*	Scaly-throated Honeyguide	0	NA	NA
467927	2	Piciformes	Indicatoridae	*Indicator*	*variegatus*	*variegatus*	Scaly-throated Honeyguide	*Plasmodium* sp.	P_BUL07	KM056642
467939	1	Piciformes	Picidae	*Campethera*	*abingoni*	*suahelica*	Golden-tailed Woodpecker	*Parahaemoproteus* sp.	H_AFR71	KM056462
467939	1	Piciformes	Picidae	*Campethera*	*abingoni*	*suahelica*	Golden-tailed Woodpecker	*Leucocytozoon* sp.	L_AFR228	KM056542
467942	1	Piciformes	Picidae	*Dendropicos*	*fuscescens*	*camacupae*	Cardinal Woodpecker	0	NA	NA
467941	1	Piciformes	Picidae	*Dendropicos*	*fuscescens*	*camacupae*	Cardinal Woodpecker	*Plasmodium* sp.	P_PSEGRI01	KM056637
467934	1	Piciformes	Ramphastidae	*Lybius*	*torquatus*	*pumilio*	Black-collared Barbet	*Parahaemoproteus* sp.	H_AFR71	KM056462
467933	1	Piciformes	Ramphastidae	*Lybius*	*torquatus*	*pumilio*	Black-collared Barbet	0	NA	NA
467932	2	Piciformes	Ramphastidae	*Pogoniulus*	*leucomystax*	*leucomystax*	Moustached Green-Tinkerbird	Unknown	Coinfection	NA
467931	2	Piciformes	Ramphastidae	*Pogoniulus*	*leucomystax*	*leucomystax*	Moustached Green-Tinkerbird	*Parahaemoproteus* sp.	H_AFR133	KM056431
467930	2	Piciformes	Ramphastidae	*Pogoniulus*	*leucomystax*	*leucomystax*	Moustached Green-Tinkerbird	0	NA	NA
467935	1	Piciformes	Ramphastidae	*Trachyphonus*	*vaillantii*	*suahelicus*	Crested Barbet	*Parahaemoproteus* sp.	H_AFR2	KM056443
467872	1	Psittaciformes	Psittacidae	*Poicephalus*	*meyeri*	*matschiei*	Meyer's Parrot	*Plasmodium* sp.	P_MALNI02	KM056641
467897	2	Trogoniformes	Trogonidae	*Heterotrogon*	*vittatus*	*vittatus*	Bar-tailed Trogon	Unknown	Coinfection	NA
467897	2	Trogoniformes	Trogonidae	*Heterotrogon*	*vittatus*	*vittatus*	Bar-tailed Trogon	*Parahaemoproteus* sp.	H_AFR138	KM056433
467896	2	Trogoniformes	Trogonidae	*Heterotrogon*	*vittatus*	*vittatus*	Bar-tailed Trogon	*Leucocytozoon* sp.	L_AFR202	KM056520
467896	2	Trogoniformes	Trogonidae	*Heterotrogon*	*vittatus*	*vittatus*	Bar-tailed Trogon	*Plasmodium* sp.	P_BUL07	KM056642

* Site 1: Malawi; Rumphi District; Vwaza Wildlife Reserve, 11˚ 08.033' S, 33˚ 39.307' E, 1071 – 1170 m

Site 2: Malawi; Rumphi District; Nyika National Park, 10˚ 35.307' S, 33˚ 48.670' E, 1647 – 2347 m

NA = Collected between sites 1 and 2

** 0 = Uninfected; no infection detected after five PCR screens

APPENDIX F

HOST SPECIES AND LIFE HISTORY TRAITS

Order	Family	Genus	Species	Subspecies	Common Name	Nest Type	Nest Location	Flocking Behavior	Habitat
Anseriformes	Anatidae	*Alopochen*	*aegyptiaca*		Egyptian Goose	1	1	2	4
Anseriformes	Anatidae	*Sarkidiornis*	*melanotos*	*melanotos*	Knob-billed Duck	3	3	2	4
Bucerotiformes	Bucerotidae	*Tockus*	*alboterminatus*	*suahelicus*	Crowned Hornbill	3	3	1	1
Bucerotiformes	Bucerotidae	*Tockus*	*nasutus*	*epirhinus*	African Gray Hornbill	3	3	1	3
Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*pectoralis*	*fervidus*	Fiery-necked Nightjar	1	1	1	3
Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*ruwenzorii*	*guttifer*	Montane Nightjar	1	1	1	3
Caprimulgiformes	Caprimulgidae	*Caprimulgus*	*fossii*	*welwitschii*	Square-tailed Nightjar	1	1	1	2
Ciconiiformes	Ardeidae	*Ardea*	*melanocephala*		Black-headed Heron	1	3	1	4
Ciconiiformes	Ardeidae	*Bubulcus*	*ibis*		Cattle Egret	1	3	2	2
Ciconiiformes	Scopidae	*Scopus*	*umbretta*		Hamerkop	1	3	1	4
Coliiformes	Coliidae	*Colius*	*striatus*	*berlepschi*	Speckled Mousebird	1	2	2	3
Columbiformes	Columbidae	*Columba*	*arquatrix*		Rameron Pigeon	1	3	2	3
Columbiformes	Columbidae	*Streptopelia*	*capicola*	*tropica*	Cape Turtle-Dove	1	3	1	2
Columbiformes	Columbidae	*Streptopelia*	*semitorquata*	*semitorquata*	Red-eyed Dove	1	3	1	3
Columbiformes	Columbidae	*Treron*	*calva*	*schalowi*	African Green-Pigeon	1	3	2	5
Columbiformes	Columbidae	*Turtur*	*chalcospilos*	*chalcospilos*	Emerald-spotted Wood-Dove	1	2	1	1
Coraciiformes	Alcedinidae	*Alcedo*	*cristata*	*cristata*	Malachite Kingfisher	3	1	1	4
Coraciiformes	Alcedinidae	*Halcyon*	*senegalensis*	*cyanoleuca*	Woodland Kingfisher	3	3	1	3
Coraciiformes	Alcedinidae	*Ispidina*	*picta*	*natalensis*	African Pygmy-Kingfisher	3	1	1	1
Coraciiformes	Coraciidae	*Eurystomus*	*glaucurus*	*glaucurus*	Broad-billed Roller	3	3	1	3
Coraciiformes	Meropidae	*Merops*	*apiaster*		European Bee-eater	3	1	2	2
Coraciiformes	Meropidae	*Merops*	*pusillus*	*meridionalis*	Little Bee-eater	3	1	2	2
Cuculiformes	Cuculidae	*Centropus*	*superciliosus*	*loandae*	White-browed Coucal	1	2	1	3
Cuculiformes	Cuculidae	*Chrysococcyx*	*klaas*	*klaas*	Klaas's Cuckoo	4	5	1	3
Cuculiformes	Cuculidae	*Cuculus*	*canorus*	*gularis*	African Cuckoo	4	3	1	1
Falconiformes	Accipitridae	*Aquila*	*wahlbergi*		Wahlberg's Eagle	1	3	1	3
Falconiformes	Accipitridae	*Milvus*	*migrans*	*parasitus*	Black (Yellow-billed) Kite	1	3	1	3
Galliformes	Numididae	*Numida*	*meleagris*	*mitrata*	Helmeted Guineafowl	1	1	2	3
Galliformes	Phasianidae	*Coturnix*	*coturnix*	*africana*	Common Quail	1	1	1	2
Galliformes	Phasianidae	*Francolinus*	*levaillantii*	*crawshayi*	Red-winged Francolin	1	1	1	2
Galliformes	Phasianidae	*Gallus*	*gallus*		Chicken	0	0	0	0
Gruiformes	Rallidae	*Sarothrura*	*rufa*	*rufa*	Red-chested Flufftail	1	1	1	2
Musophagiformes	Musophagidae	*Tauraco*	*corythaix*	*schalowi*	Schalow's Turaco	1	3	1	2
Passeriformes	Alaudidae	*Mirafra*	*rufocinnamomea*	*fischeri*	Flappet Lark	2	1	1	1
Passeriformes	Cisticolidae	*Apalis*	*thoracica*	*youngi*	Bar-throated Apalis	2	2	1	5
Passeriformes	Cisticolidae	*Calamonastes*	*undosus*	*stierlingi*	Miombo Wren-Warbler	2	2	1	1
Passeriformes	Cisticolidae	*Cisticola*	*brachyptera*	*isabellina*	Short-winged Cisticola	2	2	1	2
Passeriformes	Cisticolidae	*Cisticola*	*erythrops*	*nyasa*	Red-faced Cisticola	2	2	1	2
Passeriformes	Cisticolidae	*Cisticola*	*fulvicapilla*	*muelleri*	Piping Cisticola	2	2	1	1
Passeriformes	Cisticolidae	*Cisticola*	*natalensis*	*matengorum*	Croaking Cisticola	2	2	1	2
Passeriformes	Cisticolidae	*Cisticola*	*nigriloris*		Black-lored Cisticola	2	2	2	2
Passeriformes	Cisticolidae	*Cisticola*	*njombe**		Churring Cisticola	2	2	1	2
Passeriformes	Cisticolidae	*Cisticola*	*rufilata*	*ansorgei*	Grey Cisticola	2	2	1	1
Passeriformes	Cisticolidae	*Cisticola*	*woosnami*	*lufira*	Trilling Cisticola	2	2	1	3
Passeriformes	Cisticolidae	*Prinia*	*erythroptera*	*rhodoptera*	Red-winged Warbler	2	2	1	2
Passeriformes	Corvidae	*Corvus*	*albicollis*		White-necked Raven	1	4	1	2
Passeriformes	Dicruridae	*Dicrurus*	*adsimilis*	*adsimilis*	Fork-tailed Drongo	1	3	3	3
Passeriformes	Emberizidae	*Emberiza*	*cabanisi*	*orientalis*	Cabanis's Bunting	1	3	1	1
Passeriformes	Emberizidae	*Emberiza*	*flaviventris*	*kalaharica*	Golden-brested Bunting	1	2	1	1
Passeriformes	Estrildidae	*Sporaeginthus*	*subflavus*	*clarkei*	Zebra Waxbill	2	2	2	2
Passeriformes	Estrildidae	*Cryptospiza*	*reichenovii*	*australis*	Red-faced Crimsonwing	2	2	1	5
Passeriformes	Estrildidae	*Estrilda*	*astrild*	*cavendishi*	Common Waxbill	2	1	2	2
Passeriformes	Estrildidae	*Estrilda*	*melanotis*	*stuartirwini*	Yellow-bellied Waxbill	2	2	2	3
Passeriformes	Estrildidae	*Hypargos*	*niveoguttatus*	*macrospilotus*	Peters's Twinspot	2	2	1	3
Passeriformes	Estrildidae	*Lagonosticta*	*rubricata*	*haematocephala*	African Firefinch	2	2	1	3
Passeriformes	Estrildidae	*Lonchura*	*cucullata*	*scutata*	Bronze Mannikin	2	2	2	2
Passeriformes	Estrildidae	*Pytilia*	*afra*		Orange-winged Pytilia	2	2	1	3
Passeriformes	Estrildidae	*Pytilia*	*melba*	*melba*	Green-winged Pytilia	2	2	1	3
Passeriformes	Estrildidae	*Uraeginthus*	*angolensis*	*niassensis*	Southern Cordonbleu	2	2	2	3
Passeriformes	Eurylaimidae	*Smithornis*	*capensis*	*albigularis*	African Broadbill	2	2	1	5
Passeriformes	Fringilidae	*Serinus*	*flavivertex*	*sassii*	Yellow-crowned Canary	1	2	2	2
Passeriformes	Fringilidae	*Serinus*	*hypostictus**	*hypostictus*	Southern Citril	1	2	2	3
Passeriformes	Fringillidae	*Serinus*	*mozambicus*	*mozambicus*	Yellow-fronted Canary	1	3	3	3
Passeriformes	Fringillidae	*Serinus*	*striolatus**	*whytii*	Yellow-browed Seedeater	1	2	1	3
Passeriformes	Hirundinidae	*Delichon*	*urbica*	*urbica*	House Martin	1	4	3	2
Passeriformes	Hirundinidae	*Hirundo*	*angolensis*	*angolensis*	Angola Swallow	1	4	2	2
Passeriformes	Hirundinidae	*Psalidoprocne*	*albiceps*	*albiceps*	White-headed Sawwing	3	4	1	3
Passeriformes	Laniidae	*Lanius*	*collaris*	*capelli*	Common Fiscal	1	5	1	2
Passeriformes	Malaconotidae	*Laniarius*	*ferrugineus*	*mossambicus*	Tropical Boubou	1	5	1	3
Passeriformes	Malaconotidae	*Laniarius*	*fulleborni**	*fulleborni*	Fuelleborn's Boubou	1	2	1	5
Passeriformes	Malaconotidae	*Malaconotus*	*blanchoti*	*hypopyrrhus*	Grey-headed Bush-Shrike	1	3	1	1
Passeriformes	Malaconotidae	*Tchagra*	*australis*	*congener*	Brown-crowned Tchagra	1	2	3	3
Passeriformes	Malaconotidae	*Tchagra*	*minutus*	*anchietae*	Marsh Tchagra	1	2	1	2
Passeriformes	Malaconotidae	*Tchagra*	*senegalus*	*armenus*	Black-crowned Tchagra	1	2	1	1
Passeriformes	Monarchidae	*Terpsiphone*	*viridis*	*plumbeiceps*	African Paradise-flycatcher	1	3	3	1
Passeriformes	Monarchidae	*Trochocercus*	*albonotatus*	*albonotatus*	White-tailed Crested-Flycatcher	1	2	3	5
Passeriformes	Motacillidae	*Anthus*	*cinnamomeus*	*lichenya*	African Pipit	1	1	1	2
Passeriformes	Muscicapidae	*Cossypha*	*anomala*	*macclounii*	Olive-flanked Robin-Chat	1	2	1	5
Passeriformes	Muscicapidae	*Cossypha*	*caffra*	*iolaema*	Cape Robin-Chat	1	2	1	3
Passeriformes	Muscicapidae	*Cossypha*	*heuglini*	*heuglini*	White-browed Robin-Chat	1	2	1	3
Passeriformes	Muscicapidae	*Sheppardia*	*sharpei*	*sharpei*	Sharpe's Akalat	1	2	1	5

Passeriformes	Muscicapidae	*Erythropygia*	*barbata*		Miombo Scrub-Robin	1	2	1	1
Passeriformes	Muscicapidae	*Erythropygia*	*leucophrys*	*zambesiana*	Red-backed Scrub Robin	1	2	1	1
Passeriformes	Muscicapidae	*Pseudalethe*	*fuelleborni*		White-chested Alethe	1	2	1	5
Passeriformes	Muscicapidae	*Ficedula*	*albicollis*	*albicollis*	Collared Flycatcher	3	3	3	1
Passeriformes	Muscicapidae	*Muscicapa*	*adusta*	*subadusta*	African Dusky Flycatcher	1	3	1	3
Passeriformes	Muscicapidae	*Muscicapa*	*coerulescens*	*impavida*	Ashy Flycatcher	1	3	1	3
Passeriformes	Muscicapidae	*Myrmecocichla*	*arnotti*	*arnotti*	White-headed Black-Chat	1	3	3	1
Passeriformes	Muscicapidae	*Pogonocichla*	*stellata*	*orientalis*	White-starred Robin	2	1	1	5
Passeriformes	Muscicapidae	*Saxicola*	*torquata*	*promiscua*	Common Stonechat	1	1	1	2
Passeriformes	Nectariniidae	*Nectarinia*	*afra*	*whytei*	Montane Double-collared Sunbird	2	5	1	3
Passeriformes	Nectariniidae	*Nectarinia*	*amethystina*	*kirkii*	Amethyst Sunbird	2	3	3	3
Passeriformes	Nectariniidae	*Nectarinia*	*famosa*	*aeneigularis/ cuprea*	Malachite Sunbird	2	2	1	3
Passeriformes	Nectariniidae	*Nectarinia*	*kilimensis*	*arturi*	Bronze Sunbird	2	2	1	3
Passeriformes	Nectariniidae	*Nectarinia*	*mediocris*	*fuelleborni*	Eastern Double-collard Sunbird	2	2	3	5
Passeriformes	Nectariniidae	*Nectarinia*	*olivacea*	*alfredi*	Olive Sunbird	2	2	1	5
Passeriformes	Nectariniidae	*Nectarinia*	*senegalensis*	*gutturalis*	Scarlet-chested Sunbird	2	3	3	1
Passeriformes	Nectariniidae	*Nectarinia*	*venusta*	*falkensteini*	Variable Sunbird	2	2	1	3
Passeriformes	Oriolidae	*Oriolus*	*auratus*	*notatus*	African Golden-Oriole	1	3	3	1
Passeriformes	Paridae	*Melaniparus*	*griseiventris*		Miombo Tit	3	3	3	1
Passeriformes	Paridae	*Melaniparus*	*niger*	*niger*	Southern Black-Tit	3	3	3	1
Passeriformes	Paridae	*Parus*	*rufiventris*	*???*	Rufous-bellied Tit	3	3	3	1
Passeriformes	Passeridae	*Petronia*	*superciliaris*		Yellow-throated Petronia	3	3	3	1
Passeriformes	Passeridae	*Plocepasser*	*rufoscapulatus*		Chestnut-backed Sparrow-Weaver	2	3	2	1
Passeriformes	Pellorneidae	*Illadopsis*	*pyrrhoptera*	*nyasae*	Mountain Illadopsis	1	2	2	5
Passeriformes	Platysteiridae	*Batis*	*dimorpha*	*sola*	Malawi Batis	1	3	3	5
Passeriformes	Platysteiridae	*Batis*	*molitor*	*pallidtergum*	Chinspot Batis	1	3	3	1
Passeriformes	Ploceidae	*Euplectes*	*albonotatus*	*albonotatus*	White-winged Widowbird	2	2	3	2
Passeriformes	Ploceidae	*Euplectes*	*ardens*	*ardens*	Red-collared Widowbird	2	2	3	2
Passeriformes	Ploceidae	*Euplectes*	*capensis*	*crassirostris*	Yellow Bishop	2	2	3	2
Passeriformes	Ploceidae	*Euplectes*	*psammocromius*		Buff-shouldered Widowbird	2	2	2	2
Passeriformes	Ploceidae	*Ploceus*	*baglafecht*	*nyikae*	Baglafecht Weaver	2	5	2	3
Passeriformes	Ploceidae	*Ploceus*	*ocularis*	*suahelicus*	Spectacled Weaver	2	3	3	3
Passeriformes	Ploceidae	*Ploceus*	*velatus*	*shelleyi*	Southern Masked-Weaver	2	3	3	3
Passeriformes	Ploceidae	*Ploceus*	*xanthops*		Holub's Golden-Weaver	2	2	3	2
Passeriformes	Ploceidae	*Quelea*	*quelea*	*lathami*	Red-billed Quelea	2	5	2	2
Passeriformes	Pycnonotidae	*Phyllastrephus*	*cerviniventris*		Grey-olive Greenbul	1	2	2	1
Passeriformes	Pycnonotidae	*Phyllastrephus*	*flavostriatus*	*alfredi*	Yellow-streaked Greenbul	1	2	3	5
Passeriformes	Pycnonotidae	*Pycnonotus*	*barbatus*	*layardi*	Dark-capped Bulbul	1	3	1	3
Passeriformes	Pycnonotidae	*Arizelocichla*	*milanjensis*	*olivaceiceps*	Stripe-cheeked Greenbul	1	3	3	5
Passeriformes	Pycnonotidae	*Arizelocichla*	*nigriceps*	*fusciceps*	Eastern Mountain-Greenbul	1	2	3	5
Passeriformes	Remizidae	*Anthoscopus*	*caroli*	*robertsi*	African Penduline-Tit	2	3	1	1
Passeriformes	Stenostiridae	*Elminia*	*albonotata*	*albonotata*	White-tailed Crested-Flycatcher	1	2	3	5
Passeriformes	Sturnidae	*Lamprotornis*	*chalybaeus*	*sycobius*	Greater Blue-eared Glossy-Starling	3	3	2	3
Passeriformes	Sturnidae	*Lamprotornis*	*chloropterus*	*elisabeth*	Lesser Blue-eared Glossy-Starling	3	3	2	1
Passeriformes	Sturnidae	*Neocichla*	*gutturalis*	*angusta*	Babbling Starling	3	0	2	1
Passeriformes	Sturnidae	*Onychognathus*	*tenuirostris*	*theresae*	Slender-billed Starling	3	3	2	3
Passeriformes	Sylviidae	*Acrocephalus*	*cinnamomeus*	*cinnamomeus*	Cinnamon Bracken-Warbler	1	2	1	2
Passeriformes	Sylviidae	*Bradypterus*	*baboecala*	*tongensis*	African Bush-Warbler	1	2	1	2
Passeriformes	Sylviidae	*Bradypterus*	*cinnamomeus*	*nyassae*	Cinnamon Bracken-Warbler	1	2	1	5
Passeriformes	Sylviidae	*Iduna*	*natalensis*	*massaica*	African Yellow-Warbler	1	2	1	2
Passeriformes	Sylviidae	*Iduna*	*similis*		Mountain Yellow Warbler	1	2	1	2
Passeriformes	Sylviidae	*Phylloscopus*	*ruficapilla*	*johnstoni*	Yellow-throated Woodland-Warbler	1	1	3	5
Passeriformes	Sylviidae	*Phylloscopus*	*trochilus*		Willow Warbler	2	1	3	1
Passeriformes	Sylviidae	*Sylvia*	*abyssinica*	*stierlingi*	African Hill Babbler	1	2	1	5
Passeriformes	Sylviidae	*Sylvia*	*atricapilla*	*atricapilla*	Blackcap	1	2	3	3
Passeriformes	Sylviidae	*Sylvia*	*borin*		Garden Warbler	1	2	1	3
Passeriformes	Pellorneidae	*Illadopsis*	*pyrrhoptera*	*nyasae*	Mountain Illadopsis	1	2	2	5
Passeriformes	Turdidae	*Turdus*	*abyssinicus*	*nyikae*	Olive Thrush	1	3	1	5
Passeriformes	Turdidae	*Turdus*	*libonyanus*	*tropicalis*	Kurrichane Thrush	1	3	1	1
Passeriformes	Turdidae	*Zoothera*	*gurneyi*	*otomitra*	Orange Ground-Thrush	1	2	1	5
Passeriformes	Viduidae	*Vidua*	*macroura*		Pin-tailed Whydah	5	2	2	2
Passeriformes	Zosteropidae	*Zosterops*	*senegalensis***	*stierlingi*	Yellow White-eye	1	3	2	5
Piciformes	Indicatoridae	*Indicator*	*indicator*		Greater Honeyguide	5	5	1	3
Piciformes	Indicatoridae	*Indicator*	*minor*	*teitensis*	Lesser Honeyguide	5	5	1	3
Piciformes	Indicatoridae	*Indicator*	*variegatus*	*variegatus*	Scaly-throated Honeyguide	5	5	1	5
Piciformes	Lybiidae	*Lybius*	*torquatus*	*pumilio*	Black-collared Barbet	3	3	1	3
Piciformes	Lybiidae	*Pogoniulus*	*leucomystax*		Moustached Tinkerbird	3	3	3	5
Piciformes	Lybiidae	*Trachyphonus*	*vaillantii*	*suahelicus*	Crested Barbet	3	3	3	3
Piciformes	Picidae	*Campethera*	*abingoni*	*suahelica*	Golden-tailed Woodpecker	3	2	3	1
Piciformes	Picidae	*Dendropicos*	*fuscescens*	*camacupae*	Cardinal Woodpecker	3	2	3	1
Psittaciformes	Psittacidae	*Poicephalus*	*meyeri*	*matschiei*	Meyer's Parrot	3	3	2	1
Trogoniformes	Trogonidae	*Aploderma*	*vittatum*	*vittatum*	Bar-tailed Trogon	3	3	1	5

Nest Type: 1 = Open cup, 2 = Closed cup, 3 = Cavity, 4 = Open cup nest parasite, 5 = Cavity nest parasite
Nest Location: 1 = Ground, 2 = Understory, 3 = Canopy/subcanopy, 4 = Cliff or bank, 5 = Variable
Social System: 1 = Solitary (or in pairs), 2 = Single-species flock or family group, 3 = Mixed-species flock
Habitat: 1 = Woodland/riparian woodland, 2 = Grassland/marsh, 3 = Forest edge, 4 = Aquatic, 5 = Evergreen forest

*nest type, location, and/or habits for *Cisticola njombe*, *Serinus citrenilloides*, *Serinus striolatus*, *Laniarius fuelleborni*, and *Euplectes psammocromius* are inferred from the habits of close relatives
**Zosterops senegalensis* occurs in multiple habitat types; ours were caught in evergreen forest, so are given a 5 for habitat

APPENDIX G

SMALL MAMMAL SAMPLING (UNINFECTED INDIVIDUALS)*

Accession No.	Field No.	Species	Site**		Sex	Collection Date
Rodentia						
FMNH214746	JCK7056	*Acomys spinosissimus*	Mozambique	27	M	18-08-2011
FMNH214747	JCK7170	*Acomys spinosissimus*	Mozambique	27	M	22-08-2011
FMNH214891	JCK7114	*Aethomys* sp.	Mozambique	29	F	19-08-2011
FMNH224474	JCK8138	*Colomys goslingi*	Uganda	46	F	3-03-2013
FMNH224150	JCK8194	*Colomys goslingi*	Uganda	46	F	5-03-2013
FMNH214880	JCK6991	*Cricetomys gambianus*	Mozambique	28	F	14-08-2011
FMNH214827	JCK7113	*Cryptomys hottentotus*	Mozambique	29	F	19-08-2011
FMNH214914	JCK7115	*Cryptomys hottentotus*	Mozambique	29	F	19-08-2011
FMNH214741	JCK7000	*Dendromus melanotis*	Mozambique	30	M	15-08-2011
FMNH214742	JCK7022	*Dendromus melanotis*	Mozambique	30	F	16-08-2011
FMNH214743	JCK7023	*Dendromus melanotis*	Mozambique	30	M	16-08-2011
FMNH214740	JCK6993	*Dendromus melanotis*	Mozambique	31	M	14-08-2011
FMNH224555	JCK8701	*Dendromus mystacalis*	Uganda	48	F	6-04-2013
FMNH224464	JCK8372	*Funisciurus pyrropus*	Uganda	46	F	8-03-2013
FMNH224465	JCK8443	*Funisciurus pyrropus*	Uganda	46	F	10-03-2013
FMNH224466	JCK8444	*Funisciurus pyrropus*	Uganda	46	M	10-03-2013
FMNH224105	JCK8474	*Funisciurus pyrropus*	Uganda	46	F	11-03-2013
FMNH224467	JCK8475	*Funisciurus pyrropus*	Uganda	46	M	11-03-2013
FMNH214754	JCK7063	*Grammomys cometes*	Mozambique	27	F	18-08-2011
FMNH211572	MLWM1302	*Grammomys dolichurus*	Malawi	21	F	14-11-2009
FMNH214784	JCK7057	*Grammomys dolichurus*	Mozambique	27	M	18-08-2011
FMNH214787	JCK7086	*Grammomys dolichurus*	Mozambique	27	F	19-08-2011
FMNH214772	JCK6946	*Grammomys dolichurus*	Mozambique	28	M	12-08-2011
FMNH214902	JCK6948	*Grammomys dolichurus*	Mozambique	30	M	13-08-2011
FMNH214776	JCK6974	*Grammomys dolichurus*	Mozambique	30	F	14-08-2011
FMNH224477	JCK8494	*Grammomys dolichurus*	Uganda	46	F	12-03-2013
FMNH214728	JCK6983	*Graphiurus murinus*	Mozambique	28	M	14-08-2011
FMNH224157	JCK8132	*Hybomys lunaris*	Uganda	46	F	3-03-2013
FMNH224163	JCK8154	*Hybomys lunaris*	Uganda	46	F	3-03-2013
FMNH224164	JCK8172	*Hybomys lunaris*	Uganda	46	F	4-03-2013
FMNH224165	JCK8173	*Hybomys lunaris*	Uganda	46	F	4-03-2013
FMNH224166	JCK8174	*Hybomys lunaris*	Uganda	46	F	4-03-2013
FMNH224167	JCK8175	*Hybomys lunaris*	Uganda	46	F	4-03-2013
FMNH224173	JCK8325	*Hybomys lunaris*	Uganda	46	M	7-03-2013
FMNH224177	JCK8431	*Hybomys lunaris*	Uganda	46	M	10-03-2013
FMNH224180	JCK8473	*Hybomys lunaris*	Uganda	46	M	11-03-2013
FMNH224556	JCK8725	*Hybomys lunaris*	Uganda	47	M	9-04-2013
FMNH211578	MLWM1286	*Hylomyscus arcimontensis*	Malawi	21	M	13-11-2009
JCK8333	JCK8333	*Hylomyscus* sp.	Uganda	46	M	8-03-2013
FMNH224241	JCK8550	*Hylomyscus stella*	Uganda	44	M	17-03-2013
FMNH224243	JCK8556	*Hylomyscus stella*	Uganda	44	M	17-03-2013
FMNH224245	JCK8569	*Hylomyscus stella*	Uganda	44	F	18-03-2013
FMNH224246	JCK8570	*Hylomyscus stella*	Uganda	44	F	18-03-2013
FMNH224250	JCK8624	*Hylomyscus stella*	Uganda	44	F	20-03-2013
FMNH224251	JCK8627	*Hylomyscus stella*	Uganda	44	F	20-03-2013
FMNH224252	JCK8653	*Hylomyscus stella*	Uganda	44	F	21-03-2013
FMNH224253	JCK8655	*Hylomyscus stella*	Uganda	44	M	21-03-2013
FMNH224254	JCK8656	*Hylomyscus stella*	Uganda	44	F	21-03-2013
FMNH224255	JCK8666	*Hylomyscus stella*	Uganda	44	F	22-03-2013
FMNH223340	JCK7687	*Hylomyscus stella*	Uganda	45	F	11-11-2012
FMNH224187	JCK8167	*Hylomyscus stella*	Uganda	46	M	4-03-2013

FMNH224188	JCK8169	*Hylomyscus stella*	Uganda	46	F	4-03-2013
FMNH224190	JCK8188	*Hylomyscus stella*	Uganda	46	-	5-03-2013
FMNH224192	JCK8191	*Hylomyscus stella*	Uganda	46	M	5-03-2013
FMNH224194	JCK8195	*Hylomyscus stella*	Uganda	46	M	5-03-2013
FMNH224199	JCK8204	*Hylomyscus stella*	Uganda	46	F	5-03-2013
FMNH224200	JCK8206	*Hylomyscus stella*	Uganda	46	M	5-03-2013
FMNH224204	JCK8212	*Hylomyscus stella*	Uganda	46	F	5-03-2013
FMNH224222	JCK8331	*Hylomyscus stella*	Uganda	46	M	8-03-2013
FMNH224228	JCK8368	*Hylomyscus stella*	Uganda	46	M	8-03-2013
FMNH224232	JCK8395	*Hylomyscus stella*	Uganda	46	M	9-03-2013
FMNH224237	JCK8469	*Hylomyscus stella*	Uganda	46	F	11-03-2013
FMNH224239	JCK8492	*Hylomyscus stella*	Uganda	46	M	12-03-2013
FMNH224557	JCK8705	*Hylomyscus stella*	Uganda	47	M	7-04-2013
FMNH224558	JCK8706	*Hylomyscus stella*	Uganda	47	M	7-04-2013
FMNH224559	JCK8718	*Hylomyscus stella*	Uganda	47	M	9-04-2013
FMNH224560	JCK8738	*Hylomyscus stella*	Uganda	47	M	11-04-2013
FMNH224561	JCK8742	*Hylomyscus stella*	Uganda	47	F	12-04-2013
FMNH224562	JCK8745	*Hylomyscus stella*	Uganda	47	F	12-04-2013
FMNH224260	JCK8252	*Lemniscomys striatus*	Uganda	46	F	6-03-2013
FMNH224261	JCK8256	*Lemniscomys striatus*	Uganda	46	M	6-03-2013
FMNH224274	JCK8318	*Lemniscomys striatus*	Uganda	46	F	7-03-2013
FMNH224276	JCK8320	*Lemniscomys striatus*	Uganda	46	M	7-03-2013
FMNH224282	JCK8348	*Lemniscomys striatus*	Uganda	46	M	8-03-2013
FMNH224286	JCK8376	*Lemniscomys striatus*	Uganda	46	F	8-03-2013
FMNH224287	JCK8377	*Lemniscomys striatus*	Uganda	46	F	8-03-2013
FMNH224296	JCK8441	*Lemniscomys striatus*	Uganda	46	F	10-03-2013
FMNH224493	JCK8442	*Lemniscomys striatus*	Uganda	46	F	10-03-2013
FMNH211550	MLWM1227	*Lophuromys aquilus*	Malawi	19	F	3-11-2009
FMNH211545	MLWM1181	*Lophuromys aquilus*	Malawi	20	F	23-10-2009
FMNH211548	MLWM1201	*Lophuromys aquilus*	Malawi	20	M	29-10-2009
FMNH211549	MLWM1208	*Lophuromys aquilus*	Malawi	20	F	30-10-2009
FMNH211555	MLWM1264	*Lophuromys aquilus*	Malawi	21	F	11-11-2009
FMNH211556	MLWM1271	*Lophuromys aquilus*	Malawi	21	F	12-11-2009
FMNH211557	MLWM1279	*Lophuromys aquilus*	Malawi	21	M	13-11-2009
FMNH211558	MLWM1280	*Lophuromys aquilus*	Malawi	21	F	13-11-2009
FMNH224118	JCK8574	*Lophuromys aquilus*	Uganda	44	F	18-03-2013
FMNH224122	JCK8616	*Lophuromys aquilus*	Uganda	44	F	20-03-2013
FMNH224129	JCK8654	*Lophuromys aquilus*	Uganda	44	F	21-03-2013
FMNH224106	JCK8168	*Lophuromys aquilus*	Uganda	46	F	4-03-2013
FMNH224107	JCK8171	*Lophuromys aquilus*	Uganda	46	M	4-03-2013
FMNH224108	JCK8176	*Lophuromys aquilus*	Uganda	46	F	4-03-2013
FMNH224109	JCK8200	*Lophuromys aquilus*	Uganda	46	M	5-03-2013
FMNH224114	JCK8432	*Lophuromys aquilus*	Uganda	46	F	10-03-2013
FMNH224138	JCK8248	*Lophuromys sikapusi*	Uganda	46	F	6-03-2013
FMNH224140	JCK8254	*Lophuromys sikapusi*	Uganda	46	M	6-03-2013
FMNH224141	JCK8259	*Lophuromys sikapusi*	Uganda	46	M	6-03-2013
FMNH224142	JCK8336	*Lophuromys sikapusi*	Uganda	46	F	8-03-2013
FMNH224144	JCK8381	*Lophuromys sikapusi*	Uganda	46	M	9-03-2013
FMNH224145	JCK8440	*Lophuromys sikapusi*	Uganda	46	M	10-03-2013
FMNH224497	JCK8536	*Malacomys longipes*	Uganda	44	M	16-03-2013
FMNH224498	JCK8537	*Malacomys longipes*	Uganda	44	M	16-03-2013
FMNH224312	JCK8539	*Malacomys longipes*	Uganda	44	F	16-03-2013
FMNH224499	JCK8573	*Malacomys longipes*	Uganda	44	M	18-03-2013
FMNH224301	JCK8133	*Malacomys longipes*	Uganda	46	F	3-03-2013
FMNH224302	JCK8134	*Malacomys longipes*	Uganda	46	M	3-03-2013
FMNH224494	JCK8136	*Malacomys longipes*	Uganda	46	F	3-03-2013

FMNH224495	JCK8137	*Malacomys longipes*	Uganda	46	M	3-03-2013
FMNH224303	JCK8140	*Malacomys longipes*	Uganda	46	M	3-03-2013
FMNH224305	JCK8165	*Malacomys longipes*	Uganda	46	M	4-03-2013
FMNH224306	JCK8166	*Malacomys longipes*	Uganda	46	F	4-03-2013
FMNH224496	JCK8170	*Malacomys longipes*	Uganda	46	M	4-03-2013
FMNH224307	JCK8199	*Malacomys longipes*	Uganda	46	M	5-03-2013
FMNH224563	JCK8687	*Malacomys longipes*	Uganda	47	F	4-04-2013
FMNH224564	JCK8688	*Malacomys longipes*	Uganda	47	M	4-04-2013
FMNH224565	JCK8689	*Malacomys longipes*	Uganda	47	F	4-04-2013
FMNH224577	JCK8690	*Malacomys longipes*	Uganda	47	F	4-04-2013
FMNH224566	JCK8691	*Malacomys longipes*	Uganda	47	F	4-04-2013
FMNH224567	JCK8703	*Malacomys longipes*	Uganda	47	F	6-04-2013
FMNH224568	JCK8719	*Malacomys longipes*	Uganda	47	F	9-04-2013
FMNH224569	JCK8721	*Malacomys longipes*	Uganda	47	M	9-04-2013
FMNH224570	JCK8732	*Malacomys longipes*	Uganda	47	F	10-04-2013
FMNH224578	JCK8734	*Malacomys longipes*	Uganda	47	M	10-04-2013
FMNH214801	JCK6911	*Mastomys natalensis*	Mozambique	29	M	12-08-2011
FMNH214804	JCK6973	*Mastomys natalensis*	Mozambique	30	F	14-08-2011
FMNH224315	JCK8179	*Mus bufo*	Uganda	46	M	4-03-2013
FMNH224319	JCK8330	*Mus bufo*	Uganda	46	F	8-03-2013
FMNH224322	JCK8409	*Mus bufo*	Uganda	46	M	9-03-2013
FMNH224501	JCK8493	*Mus bufo*	Uganda	46	F	12-03-2013
FMNH214805	JCK6941	*Mus minutoides*	Mozambique	28	M	12-08-2011
FMNH214806	JCK6997	*Mus minutoides*	Mozambique	30	F	15-08-2011
FMNH224333	JCK8346	*Mus musculoides grata*	Uganda	46	M	8-03-2013
FMNH211581	MLWM1275	*Mus triton*	Malawi	21	M	12-11-2009
FMNH224345	JCK8261	*Mus triton*	Uganda	46	F	6-03-2013
FMNH224351	JCK8344	*Mus triton*	Uganda	46	F	8-03-2013
FMNH224355	JCK8382	*Mus triton*	Uganda	46	F	9-03-2013
FMNH224358	JCK8428	*Mus triton*	Uganda	46	F	10-03-2013
FMNH211630	MLWM1167	*Otomys denti sungae*	Malawi	20	F	22-10-2009
FMNH211631	MLWM1168	*Otomys denti sungae*	Malawi	20	F	22-10-2009
FMNH211632	MLWM1182	*Otomys denti sungae*	Malawi	20	F	23-10-2009
FMNH211634	MLWM1209	*Otomys denti sungae*	Malawi	20	M	30-10-2009
FMNH224455	JCK8665	*Otomys* sp.	Uganda	44	F	22-03-2013
FMNH224411	JCK8563	*Praomys jacksoni*	Uganda	44	F	18-03-2013
FMNH224415	JCK8568	*Praomys jacksoni*	Uganda	44	M	18-03-2013
FMNH224419	JCK8604	*Praomys jacksoni*	Uganda	44	F	19-03-2013
FMNH224421	JCK8657	*Praomys jacksoni*	Uganda	44	M	21-03-2013
FMNH224422	JCK8658	*Praomys jacksoni*	Uganda	44	M	21-03-2013
FMNH224402	JCK8531	*Praomys jacksoni*	Uganda	44	F	15-03-2013
FMNH224361	JCK8141	*Praomys jacksoni*	Uganda	46	M	3-03-2013
FMNH224362	JCK8143	*Praomys jacksoni*	Uganda	46	F	3-03-2013
FMNH224364	JCK8180	*Praomys jacksoni*	Uganda	46	M	4-03-2013
FMNH224366	JCK8207	*Praomys jacksoni*	Uganda	46	M	5-03-2013
FMNH224367	JCK8215	*Praomys jacksoni*	Uganda	46	F	5-03-2013
FMNH224508	JCK8274	*Praomys jacksoni*	Uganda	46	F	6-03-2013
FMNH224508	JCK8274	*Praomys jacksoni*	Uganda	46	F	6-03-2013
FMNH224378	JCK8334	*Praomys jacksoni*	Uganda	46	M	8-03-2013
FMNH224379	JCK8335	*Praomys jacksoni*	Uganda	46	F	8-03-2013
FMNH224380	JCK8337	*Praomys jacksoni*	Uganda	46	F	8-03-2013
FMNH224381	JCK8339	*Praomys jacksoni*	Uganda	46	M	8-03-2013
FMNH224386	JCK8355	*Praomys jacksoni*	Uganda	46	M	8-03-2013
FMNH224387	JCK8361	*Praomys jacksoni*	Uganda	46	M	8-03-2013
FMNH224393	JCK8393	*Praomys jacksoni*	Uganda	46	M	9-03-2013
FMNH224397	JCK8437	*Praomys jacksoni*	Uganda	46	M	10-03-2013

FMNH224398	JCK8458	*Praomys jacksoni*	Uganda	46	M	11-03-2013
FMNH224399	JCK8459	*Praomys jacksoni*	Uganda	46	M	11-03-2013
FMNH224400	JCK8461	*Praomys jacksoni*	Uganda	46	F	11-03-2013
FMNH211592	MLWM1263	*Praomys melanotus*	Malawi	21	M	11-11-2009
FMNH211593	MLWM1265	*Praomys melanotus*	Malawi	21	F	12-11-2009
FMNH211594	MLWM1266	*Praomys melanotus*	Malawi	21	M	12-11-2009
FMNH211595	MLWM1268	*Praomys melanotus*	Malawi	21	M	12-11-2009
FMNH211596	MLWM1272	*Praomys melanotus*	Malawi	21	F	12-11-2009
FMNH211597	MLWM1273	*Praomys melanotus*	Malawi	21	M	12-11-2009
FMNH211598	MLWM1274	*Praomys melanotus*	Malawi	21	M	12-11-2009
FMNH211605	MLWM1305	*Praomys melanotus*	Malawi	21	M	14-11-2009
FMNH224440	JCK8562	*Praomys misonnei*	Uganda	44	M	18-03-2013
FMNH224442	JCK8598	*Praomys misonnei*	Uganda	44	M	19-03-2013
FMNH224443	JCK8607	*Praomys misonnei*	Uganda	44	-	19-03-2013
FMNH224445	JCK8613	*Praomys misonnei*	Uganda	44	M	20-03-2013
FMNH224426	JCK8209	*Praomys misonnei*	Uganda	46	M	5-03-2013
FMNH224427	JCK8232	*Praomys misonnei*	Uganda	46	M	6-03-2013
FMNH224432	JCK8332	*Praomys misonnei*	Uganda	46	M	8-03-2013
FMNH224435	JCK8360	*Praomys misonnei*	Uganda	46	M	8-03-2013
FMNH224438	JCK8425	*Praomys misonnei*	Uganda	46	F	10-03-2013
FMNH224571	JCK8694	*Praomys* sp.	Uganda	47	F	5-04-2013
FMNH224572	JCK8720	*Praomys* sp.	Uganda	47	F	9-04-2013
FMNH224573	JCK8733	*Praomys* sp.	Uganda	47	F	10-04-2013
FMNH224574	JCK8735	*Praomys* sp.	Uganda	47	M	10-04-2013
FMNH224575	JCK8737	*Praomys* sp.	Uganda	47	F	11-04-2013
FMNH224576	JCK8739	*Praomys* sp.	Uganda	47	M	11-04-2013
JCK8527	JCK8527	*Praomys* sp.	Uganda	46	-	15-03-2013
FMNH224451	JCK8541	*Rattus rattus*	Uganda	44	F	16-03-2013
FMNH224452	JCK8542	*Rattus rattus*	Uganda	44	F	16-03-2013
FMNH224453	JCK8575	*Rattus rattus*	Uganda	44	M	18-03-2013
FMNH224454	JCK8576	*Rattus rattus*	Uganda	44	F	18-03-2013
FMNH224513	JCK8380	*Rattus rattus*	Uganda	46	M	9-03-2013
FMNH224448	JCK8460	*Rattus rattus*	Uganda	46	M	11-03-2013
FMNH224449	JCK8462	*Rattus rattus*	Uganda	46	F	11-03-2013
FMNH224450	JCK8495	*Rattus rattus*	Uganda	46	M	12-03-2013
FMNH211613	MLWM1169	*Rhabdomys dilectus*	Malawi	20	M	22-10-2009
FMNH211614	MLWM1170	*Rhabdomys dilectus*	Malawi	20	F	22-10-2009
FMNH211629	MLWM1267	*Rhabdomys dilectus*	Malawi	21	M	12-11-2009
FMNH214808	JCK7092	*Rhabdomys pumilio*	Mozambique	26	F	19-08-2011
FMNH214810	JCK7096	*Rhabdomys pumilio*	Mozambique	26	M	19-08-2011
FMNH214811	JCK7099	*Rhabdomys pumilio*	Mozambique	26	F	19-08-2011
FMNH214812	JCK7101	*Rhabdomys pumilio*	Mozambique	26	M	19-08-2011
FMNH214813	JCK7102	*Rhabdomys pumilio*	Mozambique	26	F	19-08-2011
FMNH214814	JCK7104	*Rhabdomys pumilio*	Mozambique	26	F	19-08-2011
FMNH214815	JCK7105	*Rhabdomys pumilio*	Mozambique	26	M	19-08-2011
FMNH214823	JCK7133	*Rhabdomys pumilio*	Mozambique	26	M	20-08-2011
FMNH214824	JCK7150	*Rhabdomys pumilio*	Mozambique	26	M	21-08-2011
FMNH214826	JCK7187	*Rhabdomys pumilio*	Mozambique	26	F	23-08-2011
FMNH214881	JCK6947	*Saccostomus campestris*	Mozambique	30	M	13-08-2011

Soricomorpha

FMNH223959	JCK8276	*Crocidura dolichura*	Uganda	46	M	6-03-2013
FMNH223966	JCK8651	*Crocidura hildegardeae*	Uganda	44	M	21-03-2013
FMNH223962	JCK8465	*Crocidura hildegardeae*	Uganda	46	M	11-03-2013
FMNH223963	JCK8466	*Crocidura hildegardeae*	Uganda	46	F	11-03-2013

FMNH226075	MLWM1380	*Crocidura hirta*	Malawi	24	-	16-02-2011
FMNH224515	JCK8700	*Crocidura littoralis*	Uganda	47	M	6-04-2013
FMNH224516	JCK8724	*Crocidura littoralis*	Uganda	47	F	9-04-2013
FMNH224517	JCK8730	*Crocidura littoralis*	Uganda	47	M	10-04-2013
FMNH224518	JCK8731	*Crocidura littoralis*	Uganda	47	M	10-04-2013
FMNH224519	JCK8741	*Crocidura littoralis*	Uganda	47	M	12-04-2013
FMNH224520	JCK8744	*Crocidura littoralis*	Uganda	47	M	12-04-2013
FMNH211426	MLWM1254	*Crocidura luna*	Malawi	21	M	10-11-2009
FMNH211428	MLWM1270	*Crocidura luna*	Malawi	21	M	12-11-2009
FMNH211429	MLWM1278	*Crocidura luna*	Malawi	21	M	13-11-2009
FMNH211430	MLWM1282	*Crocidura luna*	Malawi	21	M	13-11-2009
FMNH211431	MLWM1283	*Crocidura luna*	Malawi	21	F	13-11-2009
FMNH211434	MLWM1289	*Crocidura luna*	Malawi	21	M	13-11-2009
FMNH211436	MLWM1304	*Crocidura luna*	Malawi	21	M	14-11-2009
FMNH214601	JCK7172	*Crocidura luna*	Mozambique	27	M	22-08-2011
FMNH214579	JCK6872	*Crocidura luna*	Mozambique	31	F	10-08-2011
FMNH214585	JCK6913	*Crocidura luna*	Mozambique	31	F	12-08-2011
FMNH214597	JCK6996	*Crocidura luna*	Mozambique	31	M	14-08-2011
FMNH223968	JCK8384	*Crocidura luna*	Uganda	46	M	9-03-2013
FMNH223969	JCK8522	*Crocidura luna*	Uganda	46	F	13-03-2013
FMNH223971	JCK8163	*Crocidura maurisca*	Uganda	46	F	4-03-2013
FMNH223972	JCK8304	*Crocidura maurisca*	Uganda	46	M	7-03-2013
FMNH226081	MLWM1381	*Crocidura nigrofusca*	Malawi	24	-	16-02-2011
FMNH223973	JCK8265	*Crocidura nigrofusca*	Uganda	46	F	6-03-2013
FMNH223973	JCK8265	*Crocidura nigrofusca*	Uganda	46	F	6-03-2013
FMNH223974	JCK8383	*Crocidura nigrofusca*	Uganda	46	M	9-03-2013
FMNH223976	JCK8418	*Crocidura nigrofusca*	Uganda	46	M	10-03-2013
FMNH223977	JCK8430	*Crocidura nigrofusca*	Uganda	46	M	10-03-2013
FMNH223978	JCK8457	*Crocidura nigrofusca*	Uganda	46	M	11-03-2013
FMNH224522	JCK8743	*Crocidura nigrofusca*	Uganda	47	M	12-04-2013
FMNH224521	JCK8686	*Crocidura nigrofusca*	Uganda	48	M	3-04-2013
FMNH214608	JCK7121	*Crocidura olivieri*	Mozambique	27	M	20-08-2011
FMNH224456	JCK8540	*Crocidura olivieri*	Uganda	44	F	16-03-2013
FMNH224524	JCK8696	*Crocidura olivieri*	Uganda	47	F	5-04-2013
FMNH224525	JCK8697	*Crocidura olivieri*	Uganda	47	M	5-04-2013
FMNH224526	JCK8702	*Crocidura olivieri*	Uganda	47	M	6-04-2013
FMNH224527	JCK8707	*Crocidura olivieri*	Uganda	47	M	7-04-2013
FMNH224528	JCK8717	*Crocidura olivieri*	Uganda	47	M	9-04-2013
FMNH224529	JCK8723	*Crocidura olivieri*	Uganda	47	F	9-04-2013
FMNH224530	JCK8740	*Crocidura olivieri*	Uganda	47	F	11-04-2013
FMNH214618	JCK6950	*Crocidura silacea*	Mozambique	31	M	13-08-2011
MLWM1399	MLWM1399	*Crocidura sp.*	Malawi	24	-	16-02-2011
FMNH223982	JCK8603	*Crocidura* sp.	Uganda	44	M	19-03-2013
FMNH223981	JCK8162	*Crocidura* sp.	Uganda	46	M	4-03-2013
FMNH211444	MLWM1200	*Myosorex gnoskei*	Malawi	20	M	29-10-2009
FMNH214669	JCK7098	*Myosorex* sp.	Mozambique	26	M	19-08-2011
FMNH214685	JCK7149	*Myosorex* sp.	Mozambique	26	F	21-08-2011
FMNH214690	JCK7189	*Myosorex* sp.	Mozambique	26	M	23-08-2011
FMNH214665	JCK7087	*Myosorex* sp.	Mozambique	27	F	19-08-2011
FMNH214675	JCK7118	*Myosorex* sp.	Mozambique	27	F	20-08-2011
FMNH214676	JCK7123	*Myosorex* sp.	Mozambique	27	M	20-08-2011
FMNH223983	JCK8125	*Scutisorex somereni*	Uganda	46	M	3-03-2013
FMNH224531	JCK8685	*Scutisorex somereni*	Uganda	47	F	3-04-2013
FMNH224532	JCK8722	*Scutisorex somereni*	Uganda	47	M	9-04-2013
FMNH223984	JCK8548	*Suncus hututsi*	Uganda	44	M	17-03-2013
FMNH211457	MLWM1145	*Suncus megalura*	Malawi	22	M	22-10-2009

FMNH223986	JCK8490	*Suncus megalura*	Uganda	46	F	12-03-2013
FMNH224533	JCK8692	*Suncus megalura*	Uganda	48	F	5-04-2013
FMNH224534	JCK8693	*Suncus megalura*	Uganda	48	M	5-04-2013
FMNH211461	MLWM1144	*Suncus minor*	Malawi	22	F	22-10-2009
FMNH211462	MLWM1146	*Suncus minor*	Malawi	22	M	22-10-2009
FMNH224019	JCK8664	*Sylvisorex johnstoni*	Uganda	44	M	22-03-2013
FMNH223989	JCK8119	*Sylvisorex johnstoni*	Uganda	46	F	3-03-2013
FMNH223992	JCK8122	*Sylvisorex johnstoni*	Uganda	46	M	3-03-2013
FMNH223995	JCK8129	*Sylvisorex johnstoni*	Uganda	46	M	3-03-2013
FMNH223999	JCK8178	*Sylvisorex johnstoni*	Uganda	46	M	4-03-2013
FMNH224001	JCK8184	*Sylvisorex johnstoni*	Uganda	46	M	5-03-2013
FMNH224005	JCK8228	*Sylvisorex johnstoni*	Uganda	46	M	6-03-2013
FMNH224011	JCK8407	*Sylvisorex johnstoni*	Uganda	46	F	9-03-2013
FMNH224012	JCK8420	*Sylvisorex johnstoni*	Uganda	46	M	10-03-2013

Yangochiroptera

PWW2715	PWW2715	*Chaerophon pumilus*	Kenya	5	F	7-08-2014
PWW2687	PWW2687	*Chaerophon pumilus*	Kenya	2	M	1-08-2014
PWW2984	PWW2984	*Coleura afra*	Kenya	13	M	28-09-2014
PWW2985	PWW2985	*Coleura afra*	Kenya	13	F	28-09-2014
PWW2986	PWW2986	*Coleura afra*	Kenya	13	M	28-09-2014
PWW2987	PWW2987	*Coleura afra*	Kenya	13	F	28-09-2014
PWW2988	PWW2988	*Coleura afra*	Kenya	13	F	28-09-2014
PWW2982	PWW2982	*Coleura afra*	Kenya	13	F	28-09-2014
PWW2983	PWW2983	*Coleura afra*	Kenya	13	F	28-09-2014
PWW2925	PWW2925	*Glauconycteris humeralis*	Kenya	6	M	21-09-2014
PWW2685	PWW2685	*Laephotis wintoni*	Kenya	2	M	31-07-2014
PWW2969	PWW2969	*Miniopterus rufus*	Kenya	1	M	26-09-2014
PWW2972	PWW2972	*Miniopterus rufus*	Kenya	1	M	26-09-2014
PWW2973	PWW2973	*Miniopterus rufus*	Kenya	1	F	26-09-2014
PWW2978	PWW2978	*Miniopterus rufus*	Kenya	1	M	26-09-2014
PWW2952	PWW2952	*Miniopterus rufus*	Kenya	7	F	25-09-2014
PWW2954	PWW2954	*Miniopterus rufus*	Kenya	7	M	25-09-2014
PWW2955	PWW2955	*Miniopterus rufus*	Kenya	7	M	25-09-2014
PWW2961	PWW2961	*Miniopterus rufus*	Kenya	7	M	25-09-2014
PWW2962	PWW2962	*Miniopterus rufus*	Kenya	7	F	25-09-2014
PWW2734	PWW2734	*Miniopterus africanus*	Kenya	15	M	11-08-2014
PWW2740	PWW2740	*Miniopterus africanus*	Kenya	15	F	11-08-2014
PWW2741	PWW2741	*Miniopterus africanus*	Kenya	15	M	11-08-2014
PWW2742	PWW2742	*Miniopterus africanus*	Kenya	15	M	11-08-2014
PWW2743	PWW2743	*Miniopterus africanus*	Kenya	15	M	11-08-2014
PWW2744	PWW2744	*Miniopterus africanus*	Kenya	15	M	11-08-2014
PWW2739	PWW2739	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2772	PWW2772	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2773	PWW2773	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2774	PWW2774	*Miniopterus africanus*	Kenya	18	M	11-08-2014
PWW2775	PWW2775	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2777	PWW2777	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2778	PWW2778	*Miniopterus africanus*	Kenya	18	M	11-08-2014
PWW2779	PWW2779	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2780	PWW2780	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2782	PWW2782	*Miniopterus africanus*	Kenya	18	F	11-08-2014
PWW2838	PWW2838	*Miniopterus cf. fraterculus*	Kenya	14	F	13-08-2014
PWW2839	PWW2839	*Miniopterus cf. fraterculus*	Kenya	14	M	13-08-2014
PWW2840	PWW2840	*Miniopterus cf. fraterculus*	Kenya	14	F	13-08-2014

PWW2917	PWW2917	*Miniopterus cf. fraterculus*	Kenya	14	F	17-09-2014
FMNH198165	WTS8512	*Miniopterus minor*	Tanzania	40	M	27-08-2007
FMNH198166	WTS8513	*Miniopterus minor*	Tanzania	40	F	27-08-2007
FMNH198167	WTS8514	*Miniopterus minor*	Tanzania	40	F	27-08-2007
FMNH198168	WTS8515	*Miniopterus minor*	Tanzania	40	M	27-08-2007
FMNH198163	WTS8510	*Miniopterus minor*	Tanzania	40	-	27-08-2007
FMNH198162	WTS8509	*Miniopterus minor*	Tanzania	40	-	27-08-2007
FMNH198010	WTS8400	*Miniopterus minor*	Tanzania	38	-	10-08-2007
FMNH198008	WTS8389	*Miniopterus minor*	Tanzania	38	-	10-08-2007
PWW2843	PWW2843	*Miniopterus natalensis*	Kenya	14	M	13-08-2014
PWW2844	PWW2844	*Miniopterus natalensis*	Kenya	14	M	13-08-2014
PWW2845	PWW2845	*Miniopterus natalensis*	Kenya	14	M	13-08-2014
PWW2846	PWW2846	*Miniopterus natalensis*	Kenya	14	F	13-08-2014
PWW2847	PWW2847	*Miniopterus natalensis*	Kenya	14	F	13-08-2014
PWW2848	PWW2848	*Miniopterus natalensis*	Kenya	14	M	13-08-2014
PWW2746	PWW2746	*Miniopterus natalensis*	Kenya	15	F	11-08-2014
PWW2748	PWW2748	*Miniopterus natalensis*	Kenya	15	F	11-08-2014
PWW2749	PWW2749	*Miniopterus natalensis*	Kenya	15	M	11-08-2014
PWW2754	PWW2754	*Miniopterus natalensis*	Kenya	15	M	11-08-2014
PWW2755	PWW2755	*Miniopterus natalensis*	Kenya	15	M	11-08-2014
PWW2756	PWW2756	*Miniopterus natalensis*	Kenya	15	M	11-08-2014
PWW2758	PWW2758	*Miniopterus natalensis*	Kenya	15	M	11-08-2014
PWW2760	PWW2760	*Miniopterus natalensis*	Kenya	15	F	11-08-2014
PWW2761	PWW2761	*Miniopterus natalensis*	Kenya	18	F	11-08-2014
PWW2763	PWW2763	*Miniopterus natalensis*	Kenya	18	M	11-08-2014
PWW2771	PWW2771	*Miniopterus natalensis*	Kenya	18	F	11-08-2014
PWW2781	PWW2781	*Miniopterus natalensis*	Kenya	18	M	11-08-2014
PWW2783	PWW2783	*Miniopterus natalensis*	Kenya	18	M	11-08-2014
PWW2811	PWW2811	*Miniopterus natalensis*	Kenya	16	M	12-08-2014
PWW2812	PWW2812	*Miniopterus natalensis*	Kenya	16	F	12-08-2014
PWW2816	PWW2816	*Miniopterus natalensis*	Kenya	16	M	12-08-2014
PWW2989	PWW2989	*Myotis bocagii*	Kenya	12	F	28-09-2014
PWW2714	PWW2714	*Myotis tricolor*	Kenya	5	F	7-08-2014
PWW2686	PWW2686	*Myotis tricolor*	Kenya	2	F	31-07-2014
PWW2695	PWW2695	*Neoromicia capensis*	Kenya	4	M	2-08-2014
PWW2696	PWW2696	*Neoromicia capensis*	Kenya	4	F	2-08-2014
PWW2697	PWW2697	*Neoromicia capensis*	Kenya	4	F	2-08-2014
PWW2703	PWW2703	*Neoromicia capensis*	Kenya	4	F	3-08-2014
PWW2704	PWW2704	*Neoromicia capensis*	Kenya	4	M	3-08-2014
PWW2706	PWW2706	*Neoromicia capensis*	Kenya	4	M	4-08-2014
PWW2708	PWW2708	*Neoromicia capensis*	Kenya	4	M	4-08-2014
PWW2709	PWW2709	*Neoromicia capensis*	Kenya	4	M	4-08-2014
PWW2710	PWW2710	*Neoromicia capensis*	Kenya	4	F	4-08-2014
PWW2712	PWW2712	*Neoromicia capensis*	Kenya	5	F	6-08-2014
PWW2721	PWW2721	*Neoromicia capensis*	Kenya	3	F	8-08-2014
PWW2722	PWW2722	*Neoromicia capensis*	Kenya	3	F	8-08-2014
PWW2723	PWW2723	*Neoromicia capensis*	Kenya	3	M	8-08-2014
PWW2724	PWW2724	*Neoromicia capensis*	Kenya	3	M	8-08-2014
PWW2992	PWW2992	*Neoromicia capensis*	Kenya	12	F	28-09-2014
PWW3002	PWW3002	*Neoromicia capensis*	Kenya	12	F	30-09-2014
PWW2926	PWW2926	*Neoromicia nana*	Kenya	6	M	21-09-2014
PWW3004	PWW3004	*Neoromicia nana*	Kenya	12	M	1-10-2014
FMNH211530	MLWM1230	*Neoromicia nana*	Malawi	19	M	4-11-2009
FMNH211516	MLWM1172	*Neoromicia nana*	Malawi	20	M	22-10-2009
FMNH211517	MLWM1173	*Neoromicia nana*	Malawi	20	M	22-10-2009
FMNH211518	MLWM1174	*Neoromicia nana*	Malawi	20	F	22-10-2009

FMNH211519	MLWM1175	*Neoromicia nana*	Malawi	20	M	22-10-2009
FMNH211520	MLWM1176	*Neoromicia nana*	Malawi	20	M	22-10-2009
FMNH211521	MLWM1177	*Neoromicia nana*	Malawi	20	M	22-10-2009
FMNH211522	MLWM1203	*Neoromicia nana*	Malawi	20	M	29-10-2009
FMNH211523	MLWM1204	*Neoromicia nana*	Malawi	20	M	29-10-2009
FMNH211524	MLWM1210	*Neoromicia nana*	Malawi	20	M	30-10-2009
FMNH211525	MLWM1211	*Neoromicia nana*	Malawi	20	M	30-10-2009
FMNH211526	MLWM1212	*Neoromicia nana*	Malawi	20	M	30-10-2009
FMNH214723	JCK6924	*Neoromicia nana*	Mozambique	29	M	12-08-2011
FMNH214725	JCK7091	*Neoromicia nana*	Mozambique	29	F	19-08-2011
PWW2922	PWW2922	*Neoromicia* sp.	Kenya	9	M	19-09-2014
PWW2928	PWW2928	*Neoromicia* sp.	Kenya	6	F	21-09-2014
PWW2929	PWW2929	*Neoromicia* sp.	Kenya	6	F	22-09-2014
FMNH208058	WTS10123	*Neoromicia* sp.	Tanzania	35	F	19-08-2009
FMNH208060	WTS10126	*Neoromicia* sp.	Tanzania	35	F	19-08-2009
FMNH208061	WTS10127	*Neoromicia* sp.	Tanzania	35	M	19-08-2009
PWW2990	PWW2990	*Neoromicia tenuipinnis*	Kenya	12	M	28-09-2014
PWW2993	PWW2993	*Neoromicia tenuipinnis*	Kenya	12	M	28-09-2014
PWW2994	PWW2994	*Neoromicia tenuipinnis*	Kenya	12	M	28-09-2014
PWW2998	PWW2998	*Neoromicia tenuipinnis*	Kenya	12	M	29-09-2014
PWW3000	PWW3000	*Neoromicia tenuipinnis*	Kenya	12	F	30-09-2014
PWW3001	PWW3001	*Neoromicia tenuipinnis*	Kenya	12	F	30-09-2014
PWW3003	PWW3003	*Neoromicia tenuipinnis*	Kenya	12	F	1-10-2014
PWW3005	PWW3005	*Neoromicia tenuipinnis*	Kenya	12	F	1-10-2014
FMNH224102	JCK8497	*Nycteris arge*	Uganda	46	F	12-03-2013
PWW2997	PWW2997	*Nycteris aurita*	Kenya	12	M	29-09-2014
FMNH226237	MLWM1386	*Nycteris macrotis*	Malawi	23	-	16-02-2011
FMNH224103	JCK8417	*Nycteris* sp.	Uganda	46	F	9-03-2013
FMNH224104	JCK8448	*Nycteris* sp.	Uganda	46	M	10-03-2013
PWW2817	PWW2817	*Nycteris thebaica*	Kenya	14	-	13-08-2014
PWW2914	PWW2914	*Nycteris thebaica*	Kenya	14	F	17-09-2014
PWW2915	PWW2915	*Nycteris thebaica*	Kenya	14	F	17-09-2014
PWW2694	PWW2694	*Nycteris thebaica*	Kenya	4	M	2-08-2014
PWW2716	PWW2716	*Nycteris thebaica*	Kenya	3	F	8-08-2014
FMNH226238	MLWM1388	*Nycteris thebaica*	Malawi	23	-	16-02-2011
FMNH214721	JCK7196	*Nycteris thebaica*	Mozambique	32	F	28-08-2011
FMNH177856	WTS6103	*Pipistrellus hesperidus fuscatus*	Tanzania	39	F	30-08-2003
FMNH171301	WTS4958	*Pipistrellus hesperidus fuscatus*	Tanzania	42	M	2-08-2001
FMNH192531	WTS7016	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	18-07-2006
FMNH192677	WTS7026	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	18-07-2006
FMNH192678	WTS7027	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	18-07-2006
FMNH192679	WTS7028	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	18-07-2006
FMNH192680	WTS7029	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	18-07-2006
FMNH192682	WTS7030	*Pipistrellus hesperidus fuscatus*	Tanzania	37	F	18-07-2006
FMNH192681	WTS7038	*Pipistrellus hesperidus fuscatus*	Tanzania	37	M	19-07-2006
FMNH197803	WTS8047	*Pipistrellus hesperidus fuscatus*	Tanzania	41	M	26-07-2007
FMNH224554	JCK8684	*Pipistrellus hesperidus fuscatus*	Uganda	47	M	3-04-2013
PWW2991	PWW2991	*Pipistrellus rueppellii*	Kenya	12	F	28-09-2014
PWW2999	PWW2999	*Pipistrellus rueppellii*	Kenya	12	F	29-09-2014
FMNH177758	WTS6119	*Pipistrellus* sp.	Tanzania	39	M	31-08-2003
PWW2707	PWW2707	*Scotophilus dinganii*	Kenya	4	F	4-08-2014
PWW3006	PWW3006	*Scotophilus cf. leucogaster*	Kenya	12	F	1-10-2014
PWW2718	PWW2718	*Tadarida cf. lobata*	Kenya	3	M	8-08-2014
PWW2923	PWW2923	*Taphozous mauritianus*	Kenya	11	M	19-09-2014

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PWW2690	PWW2690	*Cardioderma cor*	Kenya	4	F	2-08-2014
PWW2698	PWW2698	*Cardioderma cor*	Kenya	4	F	3-08-2014
PWW2932	PWW2932	*Epomophorus labiatus*	Kenya	10	F	24-09-2014
PWW2934	PWW2934	*Epomophorus labiatus*	Kenya	10	F	24-09-2014
PWW2936	PWW2936	*Epomophorus labiatus*	Kenya	10	F	24-09-2014
PWW2937	PWW2937	*Epomophorus labiatus*	Kenya	10	F	24-09-2014
PWW2995	PWW2995	*Epomophorus labiatus*	Kenya	12	F	28-09-2014
PWW2996	PWW2996	*Epomophorus labiatus*	Kenya	12	F	28-09-2014
PWW2933	PWW2933	*Epomophorus wahlbergi*	Kenya	10	F	24-09-2014
PWW2935	PWW2935	*Epomophorus wahlbergi*	Kenya	10	F	24-09-2014
PWW2701	PWW2701	*Epomophorus wahlbergi*	Kenya	4	F	3-08-2014
PWW2705	PWW2705	*Epomophorus wahlbergi*	Kenya	4	F	3-08-2014
PWW2711	PWW2711	*Epomophorus wahlbergi*	Kenya	5	F	6-08-2014
PWW2713	PWW2713	*Epomophorus wahlbergi*	Kenya	5	F	7-08-2014
FMNH214693	JCK7038	*Epomophorus wahlbergi*	Mozambique	31	F	17-08-2011
FMNH214694	JCK7164	*Epomophorus wahlbergi*	Mozambique	31	F	22-08-2011
FMNH224024	JCK8543	*Epomops franqueti*	Uganda	44	F	17-03-2013
FMNH224461	JCK8579	*Epomops franqueti*	Uganda	44	M	18-03-2013
FMNH224026	JCK8580	*Epomops franqueti*	Uganda	44	F	18-03-2013
FMNH224027	JCK8583	*Epomops franqueti*	Uganda	44	F	19-03-2013
FMNH224035	JCK8592	*Epomops franqueti*	Uganda	44	F	19-03-2013
FMNH224036	JCK8593	*Epomops franqueti*	Uganda	44	F	19-03-2013
FMNH224042	JCK8632	*Epomops franqueti*	Uganda	44	F	20-03-2013
FMNH224045	JCK8635	*Epomops franqueti*	Uganda	44	F	20-03-2013
FMNH224046	JCK8636	*Epomops franqueti*	Uganda	44	M	20-03-2013
FMNH224048	JCK8638	*Epomops franqueti*	Uganda	44	-	20-03-2013
FMNH224051	JCK8642	*Epomops franqueti*	Uganda	44	F	21-03-2013
FMNH224060	JCK8661	*Epomops franqueti*	Uganda	44	F	22-03-2013
FMNH224061	JCK8662	*Epomops franqueti*	Uganda	44	F	22-03-2013
FMNH224062	JCK8663	*Epomops franqueti*	Uganda	44	M	22-03-2013
FMNH224023	JCK8535	*Epomops franqueti*	Uganda	44	-	16-03-2013
FMNH224535	JCK8698	*Epomops franqueti*	Uganda	48	M	5-04-2013
FMNH224538	JCK8714	*Epomops franqueti*	Uganda	48	F	9-04-2013
PWW2921	PWW2921	*Hipposideros beatus*	Kenya	9	F	19-09-2014
PWW2930	PWW2930	*Hipposideros beatus*	Kenya	8	M	22-09-2014
PWW2924	PWW2924	*Hipposideros beatus*	Kenya	1	M	20-09-2014
PWW2964	PWW2964	*Hipposideros ruber*	Kenya	1	F	26-09-2014
PWW2965	PWW2965	*Hipposideros ruber*	Kenya	1	F	26-09-2014
PWW2938	PWW2938	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2940	PWW2940	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2942	PWW2942	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2943	PWW2943	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2944	PWW2944	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2945	PWW2945	*Hipposideros ruber*	Kenya	7	M	25-09-2014
PWW2946	PWW2946	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2947	PWW2947	*Hipposideros ruber*	Kenya	7	F	25-09-2014
PWW2948	PWW2948	*Hipposideros ruber*	Kenya	7	M	25-09-2014
PWW2949	PWW2949	*Hipposideros ruber*	Kenya	7	M	25-09-2014
PWW2818	PWW2818	*Hipposideros caffer*	Kenya	14	F	13-08-2014
PWW2784	PWW2784	*Hipposideros caffer*	Kenya	17	F	12-08-2014
PWW2785	PWW2785	*Hipposideros caffer*	Kenya	17	F	12-08-2014
PWW2786	PWW2786	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2787	PWW2787	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2788	PWW2788	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2789	PWW2789	*Hipposideros caffer*	Kenya	17	F	12-08-2014

PWW2790	PWW2790	*Hipposideros caffer*	Kenya	17	F	12-08-2014
PWW2791	PWW2791	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2792	PWW2792	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2793	PWW2793	*Hipposideros caffer*	Kenya	17	F	12-08-2014
PWW2794	PWW2794	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2795	PWW2795	*Hipposideros caffer*	Kenya	17	M	12-08-2014
PWW2796	PWW2796	*Hipposideros caffer*	Kenya	17		12-08-2014
PWW2869	PWW2869	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2870	PWW2870	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2871	PWW2871	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2872	PWW2872	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2873	PWW2873	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2874	PWW2874	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2875	PWW2875	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2876	PWW2876	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2877	PWW2877	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2878	PWW2878	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2879	PWW2879	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2880	PWW2880	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2881	PWW2881	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2883	PWW2883	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2884	PWW2884	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2885	PWW2885	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2886	PWW2886	*Hipposideros caffer*	Kenya	17	M	16-09-2014
PWW2887	PWW2887	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2888	PWW2888	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2889	PWW2889	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2890	PWW2890	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2891	PWW2891	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2902	PWW2902	*Hipposideros caffer*	Kenya	17	F	16-09-2014
PWW2903	PWW2903	*Hipposideros caffer*	Kenya	17	F	16-09-2014
FMNH224069	JCK8476	*Hipposideros ruber*	Uganda	46	M	11-03-2013
FMNH224070	JCK8477	*Hipposideros ruber*	Uganda	46	F	11-03-2013
FMNH224071	JCK8478	*Hipposideros ruber*	Uganda	46	F	11-03-2013
FMNH224072	JCK8479	*Hipposideros ruber*	Uganda	46	F	11-03-2013
FMNH224073	JCK8480	*Hipposideros ruber*	Uganda	46	F	11-03-2013
FMNH224074	JCK8481	*Hipposideros ruber*	Uganda	46	F	11-03-2013
FMNH224075	JCK8482	*Hipposideros ruber*	Uganda	46	M	11-03-2013
FMNH224076	JCK8483	*Hipposideros ruber*	Uganda	46	M	11-03-2013
FMNH224077	JCK8498	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224078	JCK8499	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224079	JCK8500	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224080	JCK8501	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224081	JCK8502	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224082	JCK8503	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224083	JCK8504	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224084	JCK8505	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224085	JCK8506	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224086	JCK8507	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224087	JCK8508	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224088	JCK8509	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224089	JCK8510	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224090	JCK8511	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224091	JCK8512	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224092	JCK8513	*Hipposideros ruber*	Uganda	46	M	12-03-2013
FMNH224093	JCK8514	*Hipposideros ruber*	Uganda	46	F	12-03-2013

FMNH224094	JCK8515	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224095	JCK8516	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224096	JCK8517	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224097	JCK8518	*Hipposideros ruber*	Uganda	46	M	12-03-2013
FMNH224098	JCK8519	*Hipposideros ruber*	Uganda	46	-	12-03-2013
FMNH224099	JCK8520	*Hipposideros ruber*	Uganda	46	F	12-03-2013
FMNH224101	JCK8628	*Hipposideros* sp.	Uganda	44	M	20-03-2013
PWW2688	PWW2688	*Lavia frons*	Kenya	4	M	2-08-2014
PWW2689	PWW2689	*Lavia frons*	Kenya	4	M	2-08-2014
PWW2691	PWW2691	*Lavia frons*	Kenya	4	M	2-08-2014
PWW2692	PWW2692	*Lavia frons*	Kenya	4	M	2-08-2014
PWW2693	PWW2693	*Lavia frons*	Kenya	4	F	2-08-2014
PWW2699	PWW2699	*Lavia frons*	Kenya	4	M	3-08-2014
PWW2700	PWW2700	*Lavia frons*	Kenya	4	F	3-08-2014
PWW2702	PWW2702	*Lavia frons*	Kenya	4	F	3-08-2014
PWW2717	PWW2717	*Lavia frons*	Kenya	3	M	8-08-2014
PWW2719	PWW2719	*Lavia frons*	Kenya	3	M	8-08-2014
PWW2720	PWW2720	*Lavia frons*	Kenya	3	F	8-08-2014
PWW2931	PWW2931	*Micropteropus pusillus*	Kenya	10	F	24-09-2014
PWW2939	PWW2939	*Myonycteris angolensis*	Kenya	7	M	25-09-2014
PWW2941	PWW2941	*Myonycteris angolensis*	Kenya	7	M	25-09-2014
FMNH214700	JCK7156	*Myonycteris angolensis*	Mozambique	25	F	21-08-2011
FMNH214701	JCK7157	*Myonycteris angolensis*	Mozambique	25	M	21-08-2011
FMNH214876	JCK7182	*Myonycteris angolensis*	Mozambique	25	F	23-08-2011
FMNH214704	JCK7183	*Myonycteris angolensis*	Mozambique	25	F	23-08-2011
FMNH214705	JCK7184	*Myonycteris angolensis*	Mozambique	25	F	23-08-2011
FMNH214695	JCK7024	*Myonycteris angolensis*	Mozambique	28	F	16-08-2011
FMNH214697	JCK7138	*Myonycteris angolensis*	Mozambique	28	F	21-08-2011
FMNH214696	JCK7116	*Myonycteris angolensis*	Mozambique	31	F	20-08-2011
FMNH224546	JCK8716	*Myonycteris angolensis*	Uganda	47	F	9-04-2013
FMNH224543	JCK8683	*Myonycteris angolensis*	Uganda	47	F	2-04-2013
FMNH224544	JCK8708	*Myonycteris angolensis*	Uganda	47	M	7-04-2013
FMNH224545	JCK8709	*Myonycteris angolensis*	Uganda	48	F	8-04-2013
FMNH224547	JCK8727	*Myonycteris angolensis*	Uganda	48	F	10-04-2013
FMNH224065	JCK8640	*Myonycteris torquata*	Uganda	44	F	21-03-2013
FMNH224548	JCK8682	*Myonycteris torquata*	Uganda	47	F	2-04-2013
FMNH224549	JCK8710	*Myonycteris torquata*	Uganda	48	F	8-04-2013
PWW2725	PWW2725	*Rhinolophus clivosus*	Kenya	15	M	11-08-2014
PWW2849	PWW2849	*Rhinolophus clivosus*	Kenya	15	F	15-09-2014
PWW2850	PWW2850	*Rhinolophus clivosus*	Kenya	15	F	15-09-2014
PWW2852	PWW2852	*Rhinolophus clivosus*	Kenya	15	F	15-09-2014
PWW2854	PWW2854	*Rhinolophus clivosus*	Kenya	15	F	15-09-2014
PWW2855	PWW2855	*Rhinolophus clivosus*	Kenya	15	F	15-09-2014
PWW2735	PWW2735	*Rhinolophus clivosus*	Kenya	18	M	11-08-2014
PWW2736	PWW2736	*Rhinolophus clivosus*	Kenya	18	M	11-08-2014
FMNH197800	WTS8045	*Rhinolophus clivosus*	Tanzania	41	-	26-07-2007
FMNH197802	WTS8058	*Rhinolophus deckeni*	Tanzania	41	-	27-07-2007
FMNH197801	WTS8046	*Rhinolophus deckeni*	Tanzania	41	-	26-07-2007
PWW2833	PWW2833	*Rhinolophus eloquens*	Kenya	14	M	13-08-2014
PWW2834	PWW2834	*Rhinolophus eloquens*	Kenya	14	M	13-08-2014
PWW2835	PWW2835	*Rhinolophus eloquens*	Kenya	14	F	13-08-2014
PWW2836	PWW2836	*Rhinolophus eloquens*	Kenya	14	F	13-08-2014
PWW2837	PWW2837	*Rhinolophus eloquens*	Kenya	14	F	13-08-2014
PWW2853	PWW2853	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2856	PWW2856	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2857	PWW2857	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014

PWW2858	PWW2858	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2859	PWW2859	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2860	PWW2860	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2861	PWW2861	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2862	PWW2862	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2863	PWW2863	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2864	PWW2864	*Rhinolophus eloquens*	Kenya	15	M	15-09-2014
PWW2797	PWW2797	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2798	PWW2798	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2799	PWW2799	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2800	PWW2800	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2801	PWW2801	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2802	PWW2802	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2803	PWW2803	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2804	PWW2804	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2805	PWW2805	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2806	PWW2806	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2807	PWW2807	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2808	PWW2808	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2809	PWW2809	*Rhinolophus eloquens*	Kenya	16	M	12-08-2014
PWW2810	PWW2810	*Rhinolophus eloquens*	Kenya	16	F	12-08-2014
PWW2892	PWW2892	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2893	PWW2893	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2894	PWW2894	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2895	PWW2895	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2896	PWW2896	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2897	PWW2897	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2898	PWW2898	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2899	PWW2899	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2900	PWW2900	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
PWW2901	PWW2901	*Rhinolophus eloquens*	Kenya	16	F	16-09-2014
FMNH226188	MLWM1389	*Rhinolophus fumigatus*	Malawi	23	-	16-02-2011
FMNH226189	MLWM1390	*Rhinolophus fumigatus*	Malawi	23	-	16-02-2011
MLWM1385	MLWM1385	*Rhinolophus fumigatus*	Malawi	24	-	16-02-2011
FMNH214706	JCK7198	*Rhinolophus hildebrandti*	Mozambique	33	F	29-08-2011
FMNH214708	JCK7200	*Rhinolophus hildebrandti*	Mozambique	33	F	29-08-2011
FMNH214710	JCK7202	*Rhinolophus hildebrandti*	Mozambique	33	F	29-08-2011
FMNH214712	JCK7204	*Rhinolophus hildebrandti*	Mozambique	33	F	29-08-2011
FMNH214714	JCK7206	*Rhinolophus hildebrandti*	Mozambique	33	M	29-08-2011
FMNH214718	JCK7210	*Rhinolophus hildebrandti*	Mozambique	33	F	29-08-2011
PWW2819	PWW2819	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2820	PWW2820	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2821	PWW2821	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2822	PWW2822	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2823	PWW2823	*Rhinolophus landeri*	Kenya	14	M	13-08-2014
PWW2824	PWW2824	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2825	PWW2825	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2826	PWW2826	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2827	PWW2827	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2828	PWW2828	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2829	PWW2829	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2830	PWW2830	*Rhinolophus landeri*	Kenya	14	F	13-08-2014
PWW2831	PWW2831	*Rhinolophus landeri*	Kenya	14	M	13-08-2014
PWW2832	PWW2832	*Rhinolophus landeri*	Kenya	14	M	13-08-2014
PWW2904	PWW2904	*Rhinolophus landeri*	Kenya	14	M	17-09-2014
PWW2905	PWW2905	*Rhinolophus landeri*	Kenya	14	M	17-09-2014

PWW2906	PWW2906	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2907	PWW2907	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2908	PWW2908	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2909	PWW2909	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2910	PWW2910	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2911	PWW2911	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2912	PWW2912	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2913	PWW2913	*Rhinolophus landeri*	Kenya	14	F	17-09-2014
PWW2726	PWW2726	*Rhinolophus landeri*	Kenya	15	M	11-08-2014
PWW2727	PWW2727	*Rhinolophus landeri*	Kenya	15	M	11-08-2014
PWW2728	PWW2728	*Rhinolophus landeri*	Kenya	15	M	11-08-2014
PWW2729	PWW2729	*Rhinolophus landeri*	Kenya	15	F	11-08-2014
PWW2730	PWW2730	*Rhinolophus landeri*	Kenya	15	F	11-08-2014
PWW2731	PWW2731	*Rhinolophus landeri*	Kenya	15	F	11-08-2014
PWW2732	PWW2732	*Rhinolophus landeri*	Kenya	15	F	11-08-2014
PWW2733	PWW2733	*Rhinolophus landeri*	Kenya	15	F	11-08-2014
PWW2851	PWW2851	*Rhinolophus landeri*	Kenya	15	F	15-09-2014
PWW2865	PWW2865	*Rhinolophus landeri*	Kenya	15	M	15-09-2014
PWW2866	PWW2866	*Rhinolophus landeri*	Kenya	15	F	15-09-2014
PWW2867	PWW2867	*Rhinolophus landeri*	Kenya	15	F	15-09-2014
FMNH197799	WTS8034	*Rhinolophus simulator*	Tanzania	41	-	25-07-2007
FMNH208055	WTS9657	*Rhinolophus* sp.	Tanzania	36	M	26-07-2009
FMNH224551	JCK8704	*Rousettus aegyptiacus*	Uganda	48	F	7-04-2013
FMNH224552	JCK8713	*Rousettus aegyptiacus*	Uganda	48	F	9-04-2013
FMNH224553	JCK8715	*Rousettus aegyptiacus*	Uganda	48	F	9-04-2013

*See Appendux H for remaining sampling of individuals found to be infected

**See Appendix I for detailed site information

APPENDIX H

HOST AND PARASITE ASSOCIATIONS OF MAMMALS & BIRDS SAMPLED

Host Accession No.	Coll No.	Host species	Parasite species	Parasite Haplotype	Country	Site*	Sex	Collection Date
Hepatocystis								
FMNH224038	JCK8608	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA01	Uganda	44	M	19-Mar-2013
FMNH224041	JCK8631	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA01	Uganda	44	F	20-Mar-2013
FMNH224052	JCK8643	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA02	Uganda	44	F	21-Mar-2013
FMNH224043	JCK8633	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA03	Uganda	44	M	20-Mar-2013
FMNH224056	JCK8647	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA04	Uganda	44	F	21-Mar-2013
FMNH224540	JCK8728	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA05	Uganda	47	M	10-Apr-2013
FMNH224021	JCK8496	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	46	M	12-Mar-2013
FMNH224022	JCK8521	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	46	F	12-Mar-2013
FMNH224025	JCK8544	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	17-Mar-2013
FMNH224037	JCK8594	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	19-Mar-2013
FMNH224040	JCK8630	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	20-Mar-2013
FMNH224044	JCK8634	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	20-Mar-2013
FMNH224050	JCK8641	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	21-Mar-2013
FMNH224053	JCK8644	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	21-Mar-2013
FMNH224059	JCK8660	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA06	Uganda	44	F	22-Mar-2013
FMNH224032	JCK8589	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA07	Uganda	44	F	19-Mar-2013
FMNH224049	JCK8639	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA08	Uganda	44	M	20-Mar-2013
FMNH224054	JCK8645	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA09	Uganda	44	F	21-Mar-2013
FMNH224039	JCK8609	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA10	Uganda	44	M	19-Mar-2013
FMNH224537	JCK8711	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA10	Uganda	47	F	8-Apr-2013
FMNH224055	JCK8646	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA11	Uganda	44	F	21-Mar-2013
FMNH224541	JCK8729	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA12	Uganda	47	F	10-Apr-2013
FMNH224029	JCK8586	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA13	Uganda	44	F	19-Mar-2013
FMNH224460	JCK8534	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA14	Uganda	44	F	16-Mar-2013
FMNH224034	JCK8591	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA15	Uganda	44	F	19-Mar-2013
FMNH224030	JCK8587	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA16	Uganda	44	F	19-Mar-2013
FMNH224033	JCK8590	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA17	Uganda	44	F	19-Mar-2013
FMNH224462	JCK8596	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA18	Uganda	44	M	19-Mar-2013
FMNH224536	JCK8699	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA19	Uganda	48	F	6-Apr-2013
FMNH224542	JCK8736	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA20	Uganda	48	M	11-Apr-2013
FMNH224028	JCK8584	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA21	Uganda	44	F	19-Mar-2013
FMNH224057	JCK8648	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA22	Uganda	44	F	21-Mar-2013
FMNH224539	JCK8726	*Epomops franqueti*	*Hepatocystis* sp.	HEP_UGA23	Uganda	48	M	10-Apr-2013
FMNH224020	JCK8224	*Epomops franqueti*	*Hepatocystis* sp.	-	Uganda	46	F	6-Mar-2013
FMNH224058	JCK8649	*Epomops franqueti*	*Hepatocystis* sp.	-	Uganda	44	F	21-Mar-2013
FMNH224550	JCK8712	*Myonycteris torquata*	*Hepatocystis* sp.	HEP_UGA24	Uganda	48	F	8-Apr-2013
FMNH224064	JCK8585	*Myonycteris torquata*	*Hepatocystis* sp.	HEP_UGA25	Uganda	44	F	19-Mar-2013
FMNH224463	JCK8595	*Myonycteris torquata*	*Hepatocystis* sp.	HEP_UGA26	Uganda	44	M	19-Mar-2013
FMNH224063	JCK8181	*Myonycteris torquata*	*Hepatocystis* sp.	HEP_UGA27	Uganda	46	F	4-Mar-2013
Nycteria								
FMNH214707	JCK7199	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	M	29-Aug-2011
FMNH214709	JCK7201	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	F	29-Aug-2011
FMNH214711	JCK7203	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	M	29-Aug-2011
FMNH214713	JCK7205	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	F	29-Aug-2011
FMNH214715	JCK7207	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	F	29-Aug-2011
FMNH214716	JCK7208	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	F	29-Aug-2011
FMNH214717	JCK7209	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	M	29-Aug-2011
FMNH214719	JCK7211	*Rhinolophus hildebrandti*	*Nycteria* sp.	NYC_MOZ01	Mozambique	33	M	29-Aug-2011
FMNH224068	JCK8447	*Hipposideros cyclops*	*Nycteria* sp.	NYC_UGA02	Uganda	46	F	10-Mar-2013
FMNH224066	JCK8445	*Hipposideros cyclops*	*Nycteria* sp.	NYC_UGA03	Uganda	46	F	10-Mar-2013
FMNH224067	JCK8446	*Hipposideros cyclops*	*Nycteria* sp.	NYC_UGA03	Uganda	46	M	10-Mar-2013
FMNH226187	MLWM1379	*Rhinolophus fumigatus*	*Nycteria* sp.	NYC_MLW04	Malawi	24	F	15-Feb-2011
MLWM1391	MLWM1391	*Rhinolophus fumigatus*	*Nycteria* sp.	NYC_MLW04	Malawi	24	-	16-Feb-2011
Polychromophilus								
PWW2813	PWW2813	*Miniopterus natalensis*	*Polychromophilus* sp.	-	Kenya	16	M	12-Aug-2014
PWW2814	PWW2814	*Miniopterus natalensis*	*Polychromophilus* sp.	-	Kenya	16	F	12-Aug-2014
PWW2815	PWW2815	*Miniopterus natalensis*	*Polychromophilus* sp.	-	Kenya	16	F	12-Aug-2014
PWW2776	PWW2776	*Miniopterus africanus*	*Polychromophilus* sp.	-	Kenya	18	F	11-Aug-2014
PWW2916	PWW2916	*Miniopterus cf. fraterculus*	*Polychromophilus* sp.	-	Kenya	14	F	17-Sep-2014

PWW2737	PWW2737	*Myotis tricolor*	*P. murinus*	POLY_KEN01	Kenya	18	F	11-Aug-2014
PWW2977	PWW2977	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN02	Kenya	1	M	26-Sep-2014
PWW2958	PWW2958	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN02	Kenya	7	M	25-Sep-2014
PWW2757	PWW2757	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN02	Kenya	15	M	11-Aug-2014
PWW2768	PWW2768	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN02	Kenya	18	M	11-Aug-2014
PWW2769	PWW2769	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN03	Kenya	18	F	11-Aug-2014
PWW2762	PWW2762	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN04	Kenya	18	F	11-Aug-2014
PWW2957	PWW2957	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN05	Kenya	7	M	25-Sep-2014
PWW2979	PWW2979	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN06	Kenya	1	M	26-Sep-2014
PWW2745	PWW2745	*Miniopterus africanus*	*P. melanipherus*	POLY_KEN07	Kenya	15	M	11-Aug-2014
PWW2764	PWW2764	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN07	Kenya	18	F	11-Aug-2014
PWW2919	PWW2919	*Miniopterus cf. fraterculus*	*P. melanipherus*	POLY_KEN07	Kenya	14	M	17-Sep-2014
PWW2970	PWW2970	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN08	Kenya	1	M	26-Sep-2014
PWW2841	PWW2841	*Miniopterus africanus*	*P. melanipherus*	POLY_KEN08	Kenya	14	M	13-Aug-2014
PWW2981	PWW2981	*Miniopterus* sp.	*P. melanipherus*	POLY_KEN10	Kenya	13	F	28-Sep-2014
PWW2750	PWW2750	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN11	Kenya	15	M	11-Aug-2014
PWW2967	PWW2967	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	1	F	26-Sep-2014
PWW2974	PWW2974	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	1	F	26-Sep-2014
PWW2975	PWW2975	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	1	F	26-Sep-2014
PWW2980	PWW2980	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	1	F	26-Sep-2014
PWW2950	PWW2950	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	7	F	25-Sep-2014
PWW2953	PWW2953	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	7	F	25-Sep-2014
PWW2956	PWW2956	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	7	F	25-Sep-2014
PWW2960	PWW2960	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	7	M	25-Sep-2014
PWW2963	PWW2963	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN12	Kenya	7	F	25-Sep-2014
PWW2752	PWW2752	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN12	Kenya	15	M	11-Aug-2014
PWW2738	PWW2738	*Miniopterus africanus*	*P. melanipherus*	POLY_KEN12	Kenya	18	F	11-Aug-2014
PWW2951	PWW2951	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN14	Kenya	7	M	25-Sep-2014
PWW2747	PWW2747	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	15	F	11-Aug-2014
PWW2753	PWW2753	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	15	M	11-Aug-2014
PWW2759	PWW2759	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	15	F	11-Aug-2014
PWW2766	PWW2766	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	18	M	11-Aug-2014
PWW2767	PWW2767	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	18	M	11-Aug-2014
PWW2842	PWW2842	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	14	M	13-Aug-2014
PWW2918	PWW2918	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN15	Kenya	14	M	17-Sep-2014
PWW2751	PWW2751	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN16	Kenya	15	M	11-Aug-2014
PWW2966	PWW2966	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN17	Kenya	1	M	26-Sep-2014
PWW2971	PWW2971	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN17	Kenya	1	F	26-Sep-2014
PWW2927	PWW2927	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN17	Kenya	6	M	21-Sep-2014
PWW2920	PWW2920	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN18	Kenya	9	-	19-Sep-2014
PWW2968	PWW2968	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN19	Kenya	1	F	26-Sep-2014
PWW2976	PWW2976	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN19	Kenya	1	M	26-Sep-2014
PWW2959	PWW2959	*Miniopterus rufus*	*P. melanipherus*	POLY_KEN19	Kenya	7	M	25-Sep-2014
PWW2765	PWW2765	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN19	Kenya	18	F	11-Aug-2014
PWW2770	PWW2770	*Miniopterus natalensis*	*P. melanipherus*	POLY_KEN19	Kenya	18	F	11-Aug-2014
FMNH208063	WTS10107	*Miniopterus minor*	*P. melanipherus*	POLY_TAN09	Tanzania	35	F	18-Aug-2009
FMNH214878	JCK7185	*Miniopterus* sp.	*P. melanipherus*	POLY_TAN09	Mozambique	25	F	23-Aug-2011
FMNH198164	WTS8511	*Miniopterus minor*	*P. melanipherus*	POLY_TAN09	Tanzania	40	F	27-Aug-2007
FMNH205244	WTS9274	*Miniopterus minor*	*P. melanipherus*	POLY_TAN09	Tanzania	43	M	26-Aug-2008
FMNH205313	WTS9309	*Miniopterus minor*	*P. melanipherus*	POLY_TAN09	Tanzania	34	F	2-Sep-2008
FMNH205314	WTS9310	*Miniopterus minor*	*P. melanipherus*	POLY_TAN09	Tanzania	34	F	2-Sep-2008

Haemoproteus / Parahaemoproteus

FMNH467862	MLW3900	*Columba arquatrix*	*Haemoproteus* sp.	H_AFR109	Malawi	49	F	25-Oct-2009
FMNH467865	MLW3909	*Columba arquatrix*	*Haemoproteus* sp.	H_AFR112	Malawi	50	F	27-Oct-2009
FMNH467870	MLW3595	*Turtur chalcospilos*	*Haemoproteus* sp.	H_AFR44	Malawi	51	F	11-Oct-2009
FMNH468172	MLW3717	*Acrocephalus cinnamomeus*	*H. belopolskyi*	H_ARW1	Malawi	51	M	14-Oct-2009
FMNH468171	MLW3507	*Acrocephalus cinnamomeus*	*H. belopolskyi*	H_MW1	Malawi	51	M	10-Oct-2009
FMNH468617	MLW3792	*Dicrurus adsimilis*	*H. lanii*	H_RBS4	Malawi	51	-	17-Oct-2009
FMNH468309	MLW4178	*Parus niger niger*	*H. pallidus*	H_COLL2	Malawi	52	M	20-Nov-2009
FMNH468186	MLW4144	*Sylvia borin*	*H. parabelopolskyi*	H_SYBOR01	Malawi	52	M	18-Nov-2009
FMNH468170	MLW3492	*Acrocephalus cinnamomeus*	*H. payevski*	H_RW1	Malawi	51	M	10-Oct-2009
FMNH468001	MLW3782	*Pycnonotus barbatus layardi*	*H. sanguinis*	H_BUL2	Malawi	51	F	17-Oct-2009
FMNH468098	MLW4014	*Cossypha anomala*	*Parahaemoproteus* sp.	H_AFR122	Malawi	21	F	9-Nov-2009
FMNH467900	MLW4185	*Halcyon senegalensis*	*Parahaemoproteus* sp.	H_AFR151	Malawi	51	M	21-Nov-2009
FMNH467935	MLW3406	*Campethera abingoni*	*Parahaemoproteus* sp.	H_AFR2	Malawi	51	F	8-Oct-2009
FMNH468603	MLW3567	*Lamprotornis chloropterus*	*Parahaemoproteus* sp.	H_AFR41	Malawi	51	F	11-Oct-2009

FMNH467903	MLW3675	*Ispidina picta natalensis*	*Parahaemoproteus* sp.	H_AFR67	Malawi	51	F	14-Oct-2009
FMNH468319	MLW4145	*Nectarinia olivacea alfredi*	*Parahaemoproteus* sp.	H_CYAOLI05	Malawi	52	M	18-Nov-2009
FMNH468358	MLW3800	*Serinus striolatus whytii*	*Parahaemoproteus* sp.	H_PYERY01	Malawi	22	F	20-Oct-2009
FMNH468585	MLW3783	*Quelea quelea lathami*	*Parahaemoproteus* sp.	H_RBQ11	Malawi	51	M	17-Oct-2009
FMNH481427	MOZ085	*Camaroptera brevicaudata*	*Parahaemoproteus* sp.	H_AFR252	Mozambique	27	M	13 Aug 2011
FMNH481568	MOZ105	*Nectarinia olivacea alfredi*	*Parahaemoproteus* sp.	H_AFR253	Mozambique	27	M	15 Aug 2011
Plasmodium								
FMNH468594	MLW3760	*Neocichla gutturalis angusta*	*Plasmodium* sp.	P_ACCTAC01	Malawi	51	F	16-Oct-2009
FMNH468091	MLW3885	*Cossypha caffra iolaema*	*Plasmodium* sp.	P_AFR108	Malawi	20	M	23-Oct-2009
FMNH468315	MLW3903	*Parus griseiventris*	*Plasmodium* sp.	P_AFR110	Malawi	49	M	25-Oct-2009
FMNH468277	MLW3991	*Batis dimorpha sola*	*Plasmodium* sp.	P_AFR117	Malawi	53	M	6-Nov-2009
FMNH468216	MLW3453	*Cisticola natalensis*	*Plasmodium* sp.	P_AFR13	Malawi	51	M	8-Oct-2009
FMNH468213	MLW4151	*Cisticola woosnami lufira*	*Plasmodium* sp.	P_AFR143	Malawi	52	M	18-Nov-2009
FMNH468025	MLW4162	*Tchagra australis congener*	*Plasmodium* sp.	P_AFR145	Malawi	52	F	19-Oct-2009
FMNH468082	MLW4160	*Cossypha heuglini heuglini*	*Plasmodium* sp.	P_AFR254	Malawi	52	M	18-Nov-2009
FMNH468264	MLW3498	*Batis molitor pallidtergum*	*Plasmodium* sp.	P_AFR28	Malawi	51	F	10-Oct-2009
FMNH468029	MLW3775	*Laniarius ferrugineus*	*Plasmodium* sp.	P_AFR6	Malawi	51	M	17-Oct-2009
FMNH468312	MLW3645	*Parus niger niger*	*Plasmodium* sp.	P_AFR60	Malawi	51	M	13-Oct-2009
FMNH467945	MLW3678	*Mirafra rufocinnamomea*	*Plasmodium* sp.	P_AFR69	Malawi	51	M	14-Oct-2009
FMNH468593	MLW3751	*Neocichla gutturalis*	*Plasmodium* sp.	P_AFR80	Malawi	51	M	16-Oct-2009
FMNH468037	MLW3437	*Laniarius ferrugineus*	*Plasmodium* sp.	P_AFR9	Malawi	51	-	8-Oct-2009
FMNH468126	MLW3743	*Turdus abyssinicus nyikae*	*Plasmodium* sp.	P_AFTRU4	Malawi	51	M	15-Oct-2009
FMNH468038	MLW3488	*Laniarius ferrugineus*	*Plasmodium* sp.	P_BUL07	Malawi	51	M	10-Oct-2009
FMNH468348	MLW3617	*Nectarinia senegalensis*	*Plasmodium* sp.	P_CYAOLI04	Malawi	51	F	12-Oct-2009
FMNH468121	MLW4120	*Zoothera gurneyi otomitra*	*P. elongatum*	P_GRW06	Malawi	21	M	13-Nov-2009
FMNH468058	MLW4006	*Pogonocichla stellata*	*Plasmodium* sp.	P_GRW09	Malawi	21	F	9-Nov-2009
FMNH467996	MLW3543	*Pycnonotus barbatus*	*Plasmodium* sp.	P_LZFUS01	Malawi	51	M	10-Oct-2009
FMNH475044	MLW4304	*Euplectes capensis*	*Plasmodium* sp.	P_MALNI02	Malawi	24	M	18 Feb 2011
FMNH467959	MLW3828	*Anthus novaeseelandiae*	*Plasmodium* sp.	P_PBPIP1	Malawi	54	M	21-Oct-2009
FMNH468512	MLW3433	*Ploceus xanthops*	*P. megaloglobularis*	P_PYSUN1	Malawi	51	F	8-Oct-2009
FMNH474689	MLW4501	*Francolinus afer*	*Plasmodium* sp.	P_RFF1	Malawi	55	M	28 Feb 2011
FMNH468176	MLW3411	*Prinia erythroptera*	*Plasmodium* sp.	P_SYBOR11	Malawi	51	M	8-Oct-2009
FMNH468576	MLW3559	*Quelea quelea lathami*	*Plasmodium* sp.	P_WW3	Malawi	51	M	10-Oct-2009
FMNH481403	MOZ119	*Pogonocichla swynnertoni*	*Plasmodium* sp.	P_AFR156	Mozambique	27	F	16 Aug 2011
FMNH481395	MOZ151	*Pogonocichla swynnertoni*	*Plasmodium* sp.	P_AFR255	Mozambique	27	M	17 Aug 2011

*See Appendix I for detailed site information

APPENDIX I

SAMPLING AND SITE LOCALITIES

Site	Country	Region or District	Locality notes	Latitude	Longitude
1	Kenya	Kakamega	Kakamega Forest, Lirhanda Cave	0˚21'17.6"N	34˚89'85.6"E
2	Kenya	Laikipia	Ol Jogi Conservancy, Water treatment site	0˚30'39.6"N	36˚92'52.5"E
3	Kenya	Laikipia	Ol Jogi Conservancy, Pyramid Camp	0˚30'93.3"N	36˚07'60.9"E
4	Kenya	Laikipia	Ol Jogi Conservancy, Kiboko Campsite	0˚31'74.0"N	36˚91'08.7"E
5	Kenya	Laikipia	Ol Jogi Conservancy, Ol Jogi Dam	0˚32'46.5"N	36˚93'48.0"E
6	Kenya	Kakamega	Kakamega Forest, Salazar Trail	0˚33'51.3"N	34˚87'39.9"E
7	Kenya	Kakamega	Kakamega Forest, Mahiakalo Cave	0˚24'78.9"N	34˚90'62.8"E
8	Kenya	Kakamega	Kakamega Forest, Ikhondo Camp	0˚35'23.0"N	34˚86'47.4"E
9	Kenya	Kakamega	Kakamega Forest, Colobus Circuit 1	0˚35'61.0"N	34˚86'13.5"E
10	Kenya	Kakamega	Mukangu Village	0˚36'75.1"N	34˚86'98.3"E
11	Kenya	Kakamega	Mungokho Village	0˚37'48.5"N	34˚89'84.9"E
12	Kenya	Kisumu	Impala Sanctuary, State Lodge Campsite	0˚10'94.3"S	34˚74'61.3"E
13	Kenya	Kisumu	Kisumu, Kit Mikayi	0˚11'78.5"S	34˚54'09.9"E
14	Kenya	Nakuru	Soysambu Conservancy, Diatomite Cave	0˚43'01.0"S	36˚17'36.8"E
15	Kenya	Nakuru	Kariandusi Mines	0˚45'14.3"S	36˚28'19.4"E
16	Kenya	Nakuru	Gilgil, Pipeline Cave	0˚53'91.1"S	36˚29'43.1"E
17	Kenya	Nakuru	Gilgil, Jaika Cave	0˚56'37.1"S	36˚25'41.6"E
18	Kenya	Nakuru	Menengai Crater, Mau Mau Cave	0˚21'68.1"S	37˚13'73.3"E
19	Malawi	Rumphi District	Nyika National Park, below Chilinda Dam 2	10˚58'06.4"S	33˚80'79.2"E
20	Malawi	Rumphi District	Nyika National Park, Chilinda Dam	10˚58'84.4"S	33˚81'11.7"E
21	Malawi	Rumphi District	Nyika National Park, Mwenembwe Forest	10˚74'45.3"S	33˚98'23.9"E
22	Malawi	Rumphi District	Nyika National Park	10˚87'87.8"S	33˚46'26.9"E
23	Malawi	Mangochi District	Namizimu Forest Reserve	14˚18'87.5"S	35˚38'03.6"E
24	Malawi	Mangochi District	Namizimu Forest Reserve, Kwitunji Camp	14˚20'31.1"S	35˚37'56.1"E
25	Mozambique	Sofala, Gorongosa	Gorongosa National Park, cave at alpine-forest interface	18˚43'48.1"S	34˚05'16.7"E
26	Mozambique	Sofala, Gorongosa	Gorongosa National Park, alpine grassland	18˚43'91.3"S	34˚04'70.2"E
27	Mozambique	Sofala, Gorongosa	Gorongosa National Park, montane forest 1	18˚45'92.9"S	34˚05'53.8"E
28	Mozambique	Sofala, Gorongosa	Gorongosa National Park, montane forest 2	18˚46'66.5"S	34˚05'05.6"E
29	Mozambique	Sofala, Gorongosa	Gorongosa National Park, village below forest	18˚47'80.0"S	34˚07'60.0"E

30	Mozambique	Sofala, Gorongosa	Gorongosa National Park, below forest	18˚48'31.1"S	34˚04'46.4"E
31	Mozambique	Sofala, Gorongosa	Gorongosa National Park, Morombozi River, below forest	18˚48'38.0"S	34˚04'31.8"E
32	Mozambique	Sofala, Gorongosa	Gorongosa National Park, Muaredzi River, karst cave 1	18˚81'15.6"S	34˚73'67.5"E
33	Mozambique	Sofala, Gorongosa	Gorongosa National Park, Muaredzi River, karst cave 2	18˚95'46.7"S	34˚61'31.4"E
34	Tanzania	Mbeya, Mbeya	Songwe Caves	8˚50'00.0"S	33˚80'00.0"E
35	Tanzania	Arusha, Arumeru	Mt Meru, Arusha National Park, Meru Crater	3˚24'20.0"S	36˚78'73.6"E
36	Tanzania	Arusha, Arumeru	Mt Meru, Arusha National Park, Fig Tree Arch	3˚24'40.6"S	36˚82'84.5"E
37	Tanzania	Kilimanjaro, Mwanga	North Pare Mts, Kindoroko Forest Reserve	3˚76'03.9"S	37˚64'72.6"E
38	Tanzania	Tanga District	Tanga Region	5˚07'27.0"S	39˚04'84.8"E
39	Tanzania	Kigoma	Mahale Mts, Mahale National Park,	6˚10'43.3"S	29˚77'89.5"E
40	Tanzania	Zanzibar, Unguja I	Nyambiza village	6˚19'82.9"S	39˚33'24.2"E
41	Tanzania	Mpwapwa District	Rubeho Mts, Mwofwomero forest, near Chugu Peak	6˚83'37.0"S	36˚57'19.8"E
42	Tanzania	Rukwa, Sumbawanga	Mbizi Mts, Mbizi Forest Reserve	7˚86'39.0"S	31˚66'94.0"E
43	Tanzania	Mbeya, Rungwe	Poroto Mts, Ngozi Crater	8˚99'99.6"S	33˚56'19.0"E
44	Uganda	Kamwenge	Kibale Forest National Park, Mainaro	0˚35'75.1"N	30˚38'69.9"E
45	Uganda	Buikwe District	Mabira Forest Reserve, Najjembe	0˚39'92.1"N	33˚01'06.7"E
46	Uganda	Kamwenge	Kibale Forest National Park, Ngogo	0˚50'61.0"N	30˚42'60.8"E
47	Uganda	Rubirizi	Kasyoha-Kitomi Forest Reserve, Lake Kamuzuku 1	0˚26'36.4"S	30˚15'24.4"E
48	Uganda	Rubirizi	Kasyoha-Kitomi Forest Reserve, Lake Kamuzuku 2	0˚26'48.9"S	30˚15'70.8"E
49	Malawi	Rumphi	Nyika National Park, Runyina Bridge	10˚71'70.0"S	33˚66'70.0"E
50	Malawi	Rumphi	Nyika National Park, Juniper Forest	10˚75'00.0"S	35˚88'30.0"E
51	Malawi	Rumphi	Khuta maji, Vwaza Marsh, Vwaza Wildlife Reserve	10˚86'70.0"S	33˚45'00.0"E
52	Malawi	Rumphi	Nyika National Park, Thazima	10˚81'70.0"S	33˚58'30.0"E
53	Malawi	Rumphi	Chilinda Camp, Dam 1, Nyika National Park	10˚56'70.0"S	33˚80'00.0"E
54	Malawi	Rumphi	Chilinda Airstrip, Nyika National Park	10˚55'00.0"S	33˚78'30.0"E
55	Malawi	Mangochi	Mangochi Palm Forest Reserve	14˚50'00.0"S	35˚23'30.0"E

TAXON SAMPLING OF MAJOR HAEMOSPORIDIAN PARAITE LINEAGES FROM VERTEBRATE HOSTS. RED LABELS INDICATE NEW LIENAGES AND SEQUENCES COLLECTED IN THIS STUDY

Parasite Species*	Parasite Haplotype/Strain	Host (Collection number)	Locality	Genbank Accession Numbers**			
				Cytb	COI	Asl	ClpC
Polychromophilus (Mammalia: Chiroptera)							
**Polychromophilus melanipherus*	Poly_mel_hap3	*Miniopterus schreibersii*	Switzerland	JN990708	JN990714	-	JN990720
**Polychromophilus melanipherus*	Poly_mel_hap4	*Miniopterus schreibersii*	Switzerland	JN990709	JN990715	JN990726	JN990721
**Polychromophilus melanipherus*	Poly_mel_hap5	*Miniopterus schreibersii*	Switzerland	JN990710	JN990716	-	JN990722
**Polychromophilus melanipherus*	Poly_mel_hap6	*Miniopterus schreibersii*	Switzerland	JN990711	JN990717	-	-
****Polychromophilus melanipherus***	**POLY_KEN02**	***Miniopterus natalensis* (PWW_2757)**	**Kenya**	**KT750379**	**KT750452**	**KT750646**	**KT750738**
****Polychromophilus melanipherus***	**POLY_KEN03**	***Miniopterus natalensis* (PWW_2769)**	**Kenya**	**KT750382**	**KT750446**	**KT750633**	**-**
****Polychromophilus melanipherus***	**POLY_KEN04**	***Miniopterus natalensis* (PWW_2762)**	**Kenya**	**KT750380**	**KT750450**	**KT750647**	**KT750740**
****Polychromophilus melanipherus***	**POLY_KEN05**	***Miniopterus rufus* (PWW_2957)**	**Kenya**	**KT750385**	**KT750442**	**KT750637**	**KT750745**
****Polychromophilus melanipherus***	**POLY_KEN06**	***Miniopterus rufus* (PWW_2979)**	**Kenya**	**KT750386**	**KT750438**	**-**	**KT750748**
****Polychromophilus melanipherus***	**POLY_KEN10**	***Miniopterus sp.* (PWW_2981)**	**Kenya**	**KT750387**	**KT750436**	**KT750642**	**KT750749**
****Polychromophilus melanipherus***	**POLY_KEN11**	***Miniopterus natalensis* (PWW_2750)**	**Kenya**	**KT750377**	**KT750455**	**KT750629**	**-**
****Polychromophilus melanipherus***	**POLY_KEN12**	***Miniopterus africanus* (PWW_2738)**	**Kenya**	**KT750375**	**KT750457**	**KT750627**	**KT750734**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus natalensis* (PWW_2752)**	**Kenya**	**KT750400**	**KT750453**	**KT750630**	**KT750737**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2950)**	**Kenya**	**KT750410**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2953)**	**Kenya**	**KT750403**	**KT750443**	**KT750636**	**KT750744**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2956)**	**Kenya**	**KT750411**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2960)**	**Kenya**	**KT750404**	**KT750440**	**KT750639**	**KT750746**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2963)**	**Kenya**	**KT750413**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2967)**	**Kenya**	**KT750414**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2974)**	**Kenya**	**KT750416**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2975)**	**Kenya**	**KT750417**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN12**	***Miniopterus rufus* (PWW_2980)**	**Kenya**	**KT750418**	**KT750437**	**KT750641**	**-**
****Polychromophilus melanipherus***	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2747)**	**Kenya**	**KT750376**	**KT750456**	**KT750628**	**KT750735**
Polychromophilus melanipherus	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2759)**	**Kenya**	**KT750401**	**KT750451**	**KT750631**	**KT750739**
Polychromophilus melanipherus	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2766)**	**Kenya**	**KT750402**	**KT750448**	**KT750648**	**KT750742**
Polychromophilus melanipherus	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2767)**	**Kenya**	**KT750406**	**KT750447**	**-**	**KT750743**
Polychromophilus melanipherus	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2842)**	**Kenya**	**KT750408**	**-**	**-**	**-**
Polychromophilus melanipherus	**POLY_KEN15**	***Miniopterus natalensis* (PWW_2918)**	**Kenya**	**KT750409**	**-**	**-**	**-**
****Polychromophilus melanipherus***	**POLY_KEN16**	***Miniopterus natalensis* (PWW_2751)**	**Kenya**	**KT750378**	**KT750454**	**-**	**KT750736**
**Polychromophilus murinus*	Poly_mur_hap1	*Myotis daubentonii*	Switzerland	JN990712	JN990718	JN990725	JN990723
**Polychromophilus murinus*	Poly_mur_hap2	*Myotis daubentonii*	Switzerland	JN990713	JN990719	-	JN990724
****Polychromophilus sp.***	**POLY_TAN09**	***Miniopterus minor.* (WTS_10107)**	**Tanzania**	**KT750388**	**KT750433**	**KT750643**	**KT750750**
Polychromophilus sp.	**POLY_TAN09**	***Miniopterus minor* (WTS_8511)**	**Tanzania**	**KT750427**	**-**	**-**	**-**
Polychromophilus sp.	**POLY_TAN09**	***Miniopterus minor* (WTS_9274)**	**Tanzania**	**KT750428**	**KT750435**	**KT750644**	**KT750751**
Polychromophilus sp.	**POLY_TAN09**	***Miniopterus minor* (WTS_9309)**	**Tanzania**	**KT750429**	**KT750434**	**KT750645**	**-**
Polychromophilus sp.	**POLY_TAN09**	***Miniopterus minor* (WTS_9310)**	**Tanzania**	**KT750430**	**-**	**-**	**-**
Polychromophilus sp.	**POLY_TAN09**	***Miniopterus sp.* (JCK_7185)**	**Mozambique**	**KT750389**	**KT750541**	**KT750552**	**KT750651**
****Polychromophilus sp.***	**POLY_KEN14**	***Miniopterus rufus* (PWW_2951)**	**Kenya**	**KT750384**	**KT750444**	**KT750635**	**-**

Polychromophilus sp.	**POLY_KEN18**	***Miniopterus rufus* (PWW_2920)**	**Kenya**	**KT750383**	**KT750445**	**KT750634**	**-**
Polychromophilus sp.	**POLY_KEN19**	***Miniopterus natalensis* (PWW_2765)**	**Kenya**	**KT750381**	**KT750449**	**KT750632**	**KT750741**
Polychromophilus sp.	**POLY_KEN19**	***Miniopterus natalensis* (PWW_2770)**	**Kenya**	**KT750407**	**-**	**-**	**-**
Polychromophilus sp.	**POLY_KEN19**	***Miniopterus rufus* (PWW_2959)**	**Kenya**	**KT750412**	**KT750441**	**KT750638**	**-**
Polychromophilus sp.	**POLY_KEN19**	***Miniopterus rufus* (PWW_2968)**	**Kenya**	**KT750415**	**-**	**-**	**-**
Polychromophilus sp.	**POLY_KEN19**	***Miniopterus rufus* (PWW_2976)**	**Kenya**	**KT750405**	**KT750439**	**KT750640**	**KT750747**
**Polychromophilus sp.*	Poly_sp_Min_vil_G3_1	*Miniopterus villiersi (G-3-1)*	Guinea	KF159675	KF159794	-	-
**Polychromophilus sp.*	Poly_sp_Min_vil_G3_2	*Miniopterus villiersi (G-3-2)*	Guinea	KF159699	KF159795	-	KF159616
**Polychromophilus sp.*	Poly_sp_Min_vil_G3_3	*Miniopterus villiersi (G-3-3)*	Guinea	KF159681	KF159796	-	KF159642
Nycteria							
Nycteria sp.	**NYC_MLW04**	***Rhinolophus fumigatus* (MLWM_1391)**	**Malawi**	**KT750374**	**-**	**-**	**-**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7199)**	**Mozambique**	**KT750341**	**KT750540**	**KT750553**	**KT750652**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7201)**	**Mozambique**	**KT750419**	**KT750549**	**KT750554**	
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7203)**	**Mozambique**	**KT750420**	**KT750548**	**KT750551**	**KT750653**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7205)**	**Mozambique**	**KT750390**	**KT750539**	**KT750555**	**KT750654**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7207)**	**Mozambique**	**KT750391**	**KT750538**	**KT750556**	**KT750655**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7208)**	**Mozambique**	**KT750392**	**KT750537**	**KT750557**	**KT750656**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7209)**	**Mozambique**	**KT750393**	**KT750536**	**KT750558**	**KT750657**
Nycteria sp.	**NYC_MOZ01**	***Rhinolophus hildebrandti* (JCK_7211)**	**Mozambique**	**KT750394**	**KT750535**	**KT750559**	**KT750658**
****Nycteria sp.***	**NYC_UGA02**	***Hipposideros cyclops* (JCK_8445)**	**Uganda**	**KT750345**	**KT750533**	**KT750561**	**KT750660**
****Nycteria sp.***	**NYC_UGA03**	***Hipposideros cyclops* (JCK_8446)**	**Uganda**	**KT750346**	**KT750532**	**KT750562**	**KT750661**
Nycteria sp.	**NYC_UGA03**	***Hipposideros cyclops* (JCK_8447)**	**Uganda**	**KT750395**	**KT750531**	**KT750563**	**KT750662**
**Nycteria sp.*	Nyc_sp_R_alc_C9_1	*Rhinolophus alcyone (C-9-1)*	Cote d'Ivoire	KF159720	-	-	-
**Nycteria sp.*	Nyc_sp_R_lan_G3_1	*Rhinilophus landeri (G-3-1)*	Guinea	KF159690	KF159787	-	-
Hepatocystis (Mammalia: Primates)							
**Hepatocystis sp.*	NA	*Papio nubensis*	Ethiopia	AF069626	-	-	-
**Hepatocystis sp.*	M156_a	*Macaca sp.*	Thailand	GU930051	-	-	-
**Hepatocystis sp.*	MNR6	*Macaca sp. (MYA_1)*	Myanmar	GU930064	-	-	-
Hepatocystis (Mammalia: Chiroptera)							
**Hepatocystis sp.*	G-4-1	*Epomophorous buettikoferi*	Guinea	KF159701	KF159768	-	KF159636
**Hepatocystis sp.*	NA	*Pteropus hypomelanus*	Malaysia	FJ168565	FJ168565	-	-
**Hepatocystis sp.*	LDFB	*Cynopterus brachyoti*	Singapore	EU254526	EU254569	EU254671	EU254616
**Hepatocystis sp.*	MB3	*Nanonycteris veldkampii*	Guinea	EU254528	EU254571	EU254673	EU254618
**Hepatocystis sp.*	MB6	*Nanonycteris veldkampii*	Guinea	EU254527	EU254570	EU254672	EU254617
**Hepatocystis sp.*	G-1-1	*Micropteropus pusillus*	Guinea	KF159691	KF159800	-	KF159614
**Hepatocystis sp.*	G-2-3	*Micropteropus pusillus*	Guinea	KF159685	KF159766	-	KF159627
**Hepatocystis sp.*	G-3-1	*Micropteropus pusillus*	Guinea	KF159696	KF159769	-	KF159640
**Hepatocystis sp.*	G-5-2	*Micropteropus pusillus*	Guinea	KF159676	KF159776	-	KF159626
**Hepatocystis sp.*	C-7-1	*Myonycteris leptodon (C-7-1)*	Cote d'Ivoire	KF188066	KF188069	-	-

***Hepatocystis sp.*	C-8-1	*Nanonycteris veldkampii (C-8-1)*	Cote d'Ivoire	KF159684	KF159785	-	-
***Hepatocystis sp.*	L-1-1	*Nanonycteris veldkampii (L-1-1)*	Liberia	KF159698	KF159786	-	KF159631
****Hepatocystis sp.***	**HEP_UGA01**	***Epomops franqueti* (JCK_8608)**	**Uganda**	**KT750360**	**KT750519**	**KT750578**	**KT750674**
Hepatocystis sp.	**HEP_UGA01**	***Epomops franqueti* (JCK_8631)**	**Uganda**	**KT750397**	**KT750517**	**KT750579**	**KT750677**
****Hepatocystis sp.***	**HEP_UGA02**	***Epomops franqueti* (JCK_8643)**	**Uganda**	**KT750359**	**KT750515**	**KT750582**	**KT750679**
****Hepatocystis sp.***	**HEP_UGA04**	***Epomops franqueti* (JCK_8647)**	**Uganda**	**KT750364**	**KT750511**	**KT750585**	**-**
****Hepatocystis sp.***	**HEP_UGA05**	***Epomops franqueti* (JCK_8728)**	**Uganda**	**KT750367**	**KT750504**	**KT750591**	**KT750689**
****Hepatocystis sp.***	**HEP_UGA06**	***Epomops franqueti* (JCK_8496)**	**Uganda**	**KT750347**	**KT750530**	**KT750564**	**KT750663**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8521)**	**Uganda**	**KT750421**	**KT750547**	**KT750565**	**KT750664**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8544)**	**Uganda**	**KT750422**	**KT750546**	**KT750567**	**-**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8594)**	**Uganda**	**KT750423**	**KT750545**	**KT750575**	**-**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8630)**	**Uganda**	**KT750396**	**KT750518**	**-**	**KT750676**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8634)**	**Uganda**	**KT750425**	**KT750543**	**KT750580**	**-**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8641)**	**Uganda**	**KT750426**	**KT750542**	**KT750581**	**-**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8644)**	**Uganda**	**KT750398**	**KT750514**	**KT750583**	**KT750680**
Hepatocystis sp.	**HEP_UGA06**	***Epomops franqueti* (JCK_8660)**	**Uganda**	**KT750399**	**KT750509**	**-**	**KT750684**
****Hepatocystis sp.***	**HEP_UGA07**	***Epomops franqueti* (JCK_8589)**	**Uganda**	**KT750353**	**KT750524**	**KT750572**	**KT750670**
****Hepatocystis sp.***	**HEP_UGA08**	***Epomops franqueti* (JCK_8639)**	**Uganda**	**KT750361**	**KT750516**	**-**	**KT750678**
****Hepatocystis sp.***	**HEP_UGA09**	***Epomops franqueti* (JCK_8645)**	**Uganda**	**KT750362**	**KT750513**	**-**	**KT750681**
****Hepatocystis sp.***	**HEP_UGA10**	***Epomops franqueti* (JCK_8711)**	**Uganda**	**KT750366**	**KT750507**	**KT750588**	**KT750686**
Hepatocystis sp.	**HEP_UGA10**	***Epomops franqueti* (JCK_8609)**	**Uganda**	**KT750424**	**KT750544**	**-**	**KT750675**
****Hepatocystis sp.***	**HEP_UGA11**	***Epomops franqueti* (JCK_8646)**	**Uganda**	**KT750363**	**KT750512**	**KT750584**	**KT750682**
****Hepatocystis sp.***	**HEP_UGA12**	***Epomops franqueti* (JCK_8729)**	**Uganda**	**KT750368**	**KT750503**	**-**	**KT750690**
****Hepatocystis sp.***	**HEP_UGA13**	***Epomops franqueti* (JCK_8586)**	**Uganda**	**KT750351**	**KT750526**	**KT750570**	**KT750668**
****Hepatocystis sp.***	**HEP_UGA14**	***Epomops franqueti* (JCK_8534)**	**Uganda**	**KT750348**	**KT750529**	**KT750566**	**KT750665**
****Hepatocystis sp.***	**HEP_UGA15**	***Epomops franqueti* (JCK_8591)**	**Uganda**	**KT750355**	**KT750522**	**KT750574**	**KT750672**
****Hepatocystis sp.***	**HEP_UGA16**	***Epomops franqueti* (JCK_8587)**	**Uganda**	**KT750352**	**KT750525**	**KT750571**	**KT750669**
****Hepatocystis sp.***	**HEP_UGA17**	***Epomops franqueti* (JCK_8590)**	**Uganda**	**KT750354**	**KT750523**	**KT750573**	**KT750671**
****Hepatocystis sp.***	**HEP_UGA18**	***Epomops franqueti* (JCK_8596)**	**Uganda**	**KT750358**	**KT750520**	**KT750577**	**-**
****Hepatocystis sp.***	**HEP_UGA19**	***Epomops franqueti* (JCK_8699)**	**Uganda**	**KT750365**	**KT750508**	**KT750587**	**KT750685**
****Hepatocystis sp.***	**HEP_UGA20**	***Epomops franqueti* (JCK_8736)**	**Uganda**	**KT750369**	**KT750502**	**KT750592**	**KT750691**
****Hepatocystis sp.***	**HEP_UGA21**	***Epomops franqueti* (JCK_8584)**	**Uganda**	**KT750349**	**KT750528**	**KT750568**	**KT750666**
****Hepatocystis sp.***	**HEP_UGA22**	***Epomops franqueti* (JCK_8648)**	**Uganda**	**KT750350**	**KT750510**	**KT750586**	**KT750683**
****Hepatocystis sp.***	**HEP_UGA23**	***Epomops franqueti* (JCK_8726)**	**Uganda**	**KT750344**	**KT750505**	**KT750590**	**KT750688**
****Hepatocystis sp.***	**HEP_UGA24**	***Myonycteris torquata* (JCK_8712)**	**Uganda**	**KT750343**	**KT750506**	**KT750589**	**KT750687**
****Hepatocystis sp.***	**HEP_UGA25**	***Myonycteris torquata* (JCK_8585)**	**Uganda**	**KT750356**	**KT750527**	**KT750569**	**KT750667**
****Hepatocystis sp.***	**HEP_UGA26**	***Myonycteris torquata* (JCK_8595)**	**Uganda**	**KT750357**	**KT750521**	**KT750576**	**KT750673**
****Hepatocystis sp.***	**HEP_UGA27**	***Myonycteris torquata* (JCK_8181)**	**Uganda**	**KT750342**	**KT750534**	**KT750560**	**KT750659**

Plasmodium (Mammalia: Primates)

***Plasmodium coatneyi*	NA	Macacus spp.	Thailand	EU400407	AB354575	-	AB471872
***Plasmodium cynomolgi*	NA	Old World monkeys	Southeast Asia	AF069616	AB444126	-	AB471873
***Plasmodium fieldi*	NA	Old World monkeys	Malaysia	gi|195976618	AB354574	-	AB471874
***Plasmodium gonderi*	NA	Old World monkeys	Central Africa	AF069622	AB434918	-	AB471877

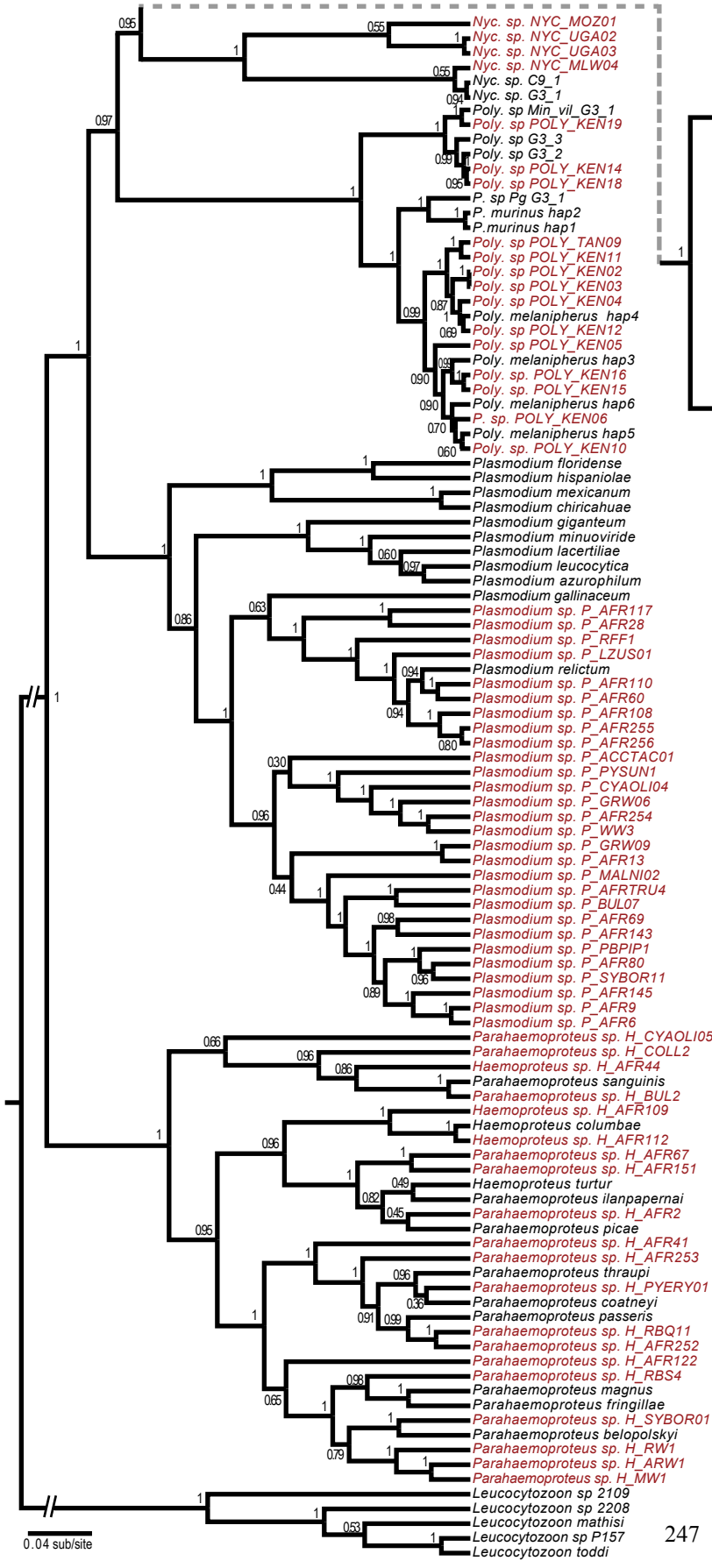
**Plasmodium hylobati*	NA	*Hylobati moloch*	Malaysia	gi|195976614	AB354573	-	AB471878
**Plasmodium inui*	NA	Old World monkeys	Asia	AF069617	AB354572	-	AB471879
**Plasmodium knowlesi*	NA	Old World monkeys	Malaysia	AF069621	AY598141	XM002257931	AF348341
**Plasmodium malariae*	NA	*Homo sapiens*	Tropics/Subtropics	AF069624	AB489193	-	AF348342
**Plasmodium ovale*	NA	*Homo sapiens*	Old World tropics	AF069625	JF894415	-	AY634623
**Plasmodium simiovale*	NA	Old World monkeys	Sri Lanka	AF069614	AY800109	KF618387	AB471881
**Plasmodium simium*	NA	*Alouatta fuscus*	Brazil	AF069620	-	-	-
**Plasmodium vivax*	NA	*Homo sapiens*	Tropics/Subtropics	AF069619	AY791540	XM001612942	AF348344
(Subgenus *Laverania*)							
**Plasmodium billbrayi*	NA	*Pan troglodytes*	Uganda	GQ355470	GQ355470	-	-
**Plasmodium billcollinsi*	NA	*Pan troglodytes*	Uganda	GQ355477	GQ355477	-	-
**Plasmodium falciparum*	P_falciparum_1	*Homo sapiens*	Tropical regions	AF069605	M76611	XM001349541	DQ642846
**Plasmodium gaboni*	P_gaboni_K	*Pan troglodytes*	Gabon	FJ895307	FJ895307	-	HQ842630
**Plasmodium reichenowi*	P_reichenowi_1	*Pan troglodytes*	Africa	AJ251941	AJ251941	AB519183	EU560464
Plasmodium (Mammalia: Rodentia)							
**Plasmodium artheruri*	R20	*Arthererus africanus*	DR Congo	EU254524	EU254568	EU254669	EU254615
**Plasmodium berghei*	ANKA	*Grammomys surdaster*	DR Congo	DQ414645	DQ414589	AF262049	DQ417612
**Plasmodium chabaudi*	408XZ	*Thamnomys rutilans*	DR Congo	DQ414647	DQ414591	-	DQ417614
**Plasmodium vinckei*	R8	*Grammomys surdaster*	DR Congo	EU254522	EU254567	EU254667	EU254613
**Plasmodium yoelii*	PY17X01			LM993670	LM993670	LM993657	LM993699
Plasmodium (Mammalia: Chrioptera)							
**Plasmodium cyclopsi*	P_sp_Hip_cy_L1_1	*Hipposideros cyclops (L-1-1)*	Liberia	KF159710	KF159788	-	KF159635
**Plasmodium voltaicum*	P_sp_M_ang_G1_1	*Myonycteris angolensis (G-1-1)*	Guinea	KF159671	KF159792	KF159654	KF159648
Plasmodium (Aves: multiple orders)							
**Plasmodium gallinaceum*	RP	*Gallus gallus*	Vietnam	EU254535	EU254578	EU254680	EU254625
**Plasmodium relictum*	B170	*Sialia mexicana*	United States	EU254538	EU254581	EU254689	EU254633
****Plasmodium sp.***	**P_AFR255**	***Pogonocichla swynnertoni* (MOZ11_151)**	**Mozambique**	**KT750373**	**KT750459**	**KT750625**	**-**
****Plasmodium sp.***	**P_AFR256**	***Pogonocichla stellata* (MOZ11_119)**	**Mozambique**	**KT750372**	**KT750460**	**KT750624**	**KT750732**
****Plasmodium sp.***	**P_AFR108, AFR208?**	***Cossypha caffra* (MLW_3885)**	**Malawi**	**KM056569**	**KT750477**	**KT750610**	**KT750716**
****Plasmodium sp.***	**P_AFR110**	***Anthoscopus caroli robertsi* (MLW_3903)**	**Malawi**	**KM056570**	**KT750476**	**KT750612**	**KT750718**
****Plasmodium sp.***	**P_AFR117**	***Batis dimorpha sola* (MLW_3991)**	**Malawi**	**KM056572**	**KT750474**	**KT750614**	**KT750720**
****Plasmodium sp.***	**P_AFR13**	***Cisticola natalensis* (MLW_3453)**	**Malawi**	**KM056579**	**KT750497**	**-**	**-**
****Plasmodium sp.***	**P_AFR143**	***Cisticola woosnami lufira* (MLW_4151)**	**Malawi**	**KM056585**	**KT750469**	**KT750618**	**KT750753**
****Plasmodium sp.***	**P_AFR145**	***Tchagra australis congener* (MLW_4162)**	**Malawi**	**KM056586**	**KT750467**	**KT750620**	**KT750725**
****Plasmodium sp.***	**P_AFR254**	***Cossypha hueglini hueglini* (MLW_4160)**	**Malawi**	**-**	**KT750468**	**KT750619**	**KT750752**
****Plasmodium sp.***	**P_AFR28**	***Batis molitor* (MLW_3498)**	**Malawi**	**KM056593**	**KT750495**	**-**	**KT750697**
****Plasmodium sp.***	**P_AFR6**	***Laniarius ferrungineus* (MLW_3775)**	**Malawi**	**KM056605**	**KT750483**	**KT750605**	**KT750710**
****Plasmodium sp.***	**P_AFR60**	***Parus niger* (MLW_3645)**	**Malawi**	**KM056606**	**KT750489**	**KT750600**	**KT750703**

Plasmodium sp.	**P_AFR69**	***Mirafra rufocinnamomea* (MLW_3678)**	**Malawi**	**KM056609**	**KT750432**	**KT750649**	**KT750705**
Plasmodium sp.	**P_AFR80**	***Neocichla gutturalis augusta* (MLW_3751)**	**Malawi**	**KM056610**	**KT750485**	**KT750603**	**KT750708**
Plasmodium sp.	**P_AFR9**	***Laniarius ferrungineus* (MLW_3437)**	**Malawi**	**KM056614**	**KT750498**	**KT750595**	**KT750695**
Plasmodium sp.	**P_AFTRU4**	***Turdus abyssinicus* (MLW_3743)**	**Malawi**	**KM056635**	**KT750486**	**KT750602**	**KT750707**
Plasmodium sp.	**P_BUL07**	***Laniarius ferrungineus* (MLW_3488)**	**Malawi**	**KM056642**	**KT750458**	**KT750626**	**KT750733**
Plasmodium sp.	**P_CYAOLI04**	***Nectarinia senegalensis* (MLW_3617)**	**Malawi**	**KM056640**	**KT750490**	**KT750599**	**KT750702**
Plasmodium sp.	**P_GRW06**	***Zoothera gurneyi otomitra* (MLW_4120)**	**Malawi**	**KM056633**	**KT750471**	**KT750616**	**-**
Plasmodium sp.	**P_GRW09**	***Pogonocichla stellata* (MLW_4006)**	**Malawi**	**KM056631**	**KT750473**	**KT750615**	**KT750721**
Plasmodium sp.	**P_LZFUS01**	***Pycnonotus barbatus* (MLW_3543)**	**Malawi**	**KM056627**	**KT750494**	**KT750597**	**KT750698**
Plasmodium sp.	**P_MALNI02**	***Euplectes capensis crassirostris* (MLW_4304)**	**Malawi**	**KM056641**	**KT750464**	**KT750621**	**KT750728**
Plasmodium sp.	**P_PBPIP1**	***Anthus novaeseelandiae* (MLW_3828)**	**Malawi**	**KM056639**	**KT750478**	**KT750609**	**KT750715**
Plasmodium sp.	**P_PYSUN1**	***Ploceus xanthops* (MLW_3433)**	**Malawi**	**KM056628**	**KT750499**	**KT750594**	**KT750694**
Plasmodium sp.	**P_RFF1**	***Francolinus afer humboldtii* (MLW_4501)**	**Malawi**	**KM056632**	**KT750463**	**KT750622**	**KT750729**
Plasmodium sp.	**P_SYBOR11**	***Prinia erythoptera* (MLW_3411)**	**Malawi**	**KM056638**	**KT750500**	**-**	**KT750693**
Plasmodium sp.	**P_WW4**	***Quelea quelea* (MLW_3559)**	**Malawi**	**KM056634**	**KT750493**	**KT750598**	**KT750699**
Plasmodium sp.	**P_ACCTAC01**	***Neocichla gutturalis augusta* (MLW_3760)**	**Malawi**	**KM056621**	**KT750484**	**KT750604**	**KT750709**

Parahaemoproteus/Haemoproteus (Aves: multiple orders)

Parahaemoproteus sp.	**H_AFR122**	***Cossypha anomala macclounii* (MLW_4014)**	**Malawi**	**KM056428**	**KT750472**	**-**	**KT750722**
Parahaemoproteus sp.	**H_AFR151**	***Halcyon senegalensis* (MLW_4185)**	**Malawi**	**KM056440**	**KT750465**	**-**	**KT750727**
Parahaemoproteus sp.	**H_AFR2**	***Campethera abingoni* (MLW_3406)**	**Malawi**	**KM056443**	**KT750501**	**KT750593**	**KT750692**
Parahaemoproteus sp.	**H_AFR41**	***Lamprotornis chloropterus* (MLW_3567)**	**Malawi**	**KM056448**	**KT750492**	**-**	**KT750700**
Parahaemoproteus sp.	**H_AFR67**	***Ispidina picta natalensis* (MLW_3675)**	**Malawi**	**KM056460**	**KT750488**	**KT750601**	**KT750704**
Haemoproteus belopolskyi	**H_ARW1**	***Acrocephalus cinnamomeus* (MLW_3717)**	**Malawi**	**KM056407**	**KT750487**	**-**	**KT750706**
Haemoproteus sanguinis	**H_BUL2**	***Pycnonotus barbatus layardi* (MLW_3782)**	**Malawi**	**KM056412**	**KT750482**	**KT750606**	**KT750711**
Haemoproteus pallidus	**H_COLL2**	***Parus niger niger* (MLW_4178)**	**Malawi**	**KM056413**	**KT750466**	**-**	**KT750726**
Parahaemoproteus sp.	**H_CYAOLI05**	***Nectarinia olivacea alfredi* (MLW_4145)**	**Malawi**	**KM056417**	**KT750431**	**-**	**KT750724**
Parahaemoproteus sp.	**H_PYERY01**	***Serinus striolatus whytii* (MLW_3800)**	**Malawi**	**KM056418**	**KT750479**	**-**	**KT750714**
Parahaemoproteus sp.	**H_RBQ11**	***Onychognathus tenuirostris* (MLW_3783)**	**Malawi**	**KM056419**	**KT750481**	**KT750607**	**KT750712**
Haemoproteus lanii	**H_RBS4**	***Dicrurus adsmilis adsimilis* (MLW_3792)**	**Malawi**	**KM056411**	**KT750480**	**KT750608**	**KT750713**
Haemoproteus payevski	**H_RW1**	***Acrocephalus cinnamomeus* (MLW_3492)**	**Malawi**	**KM056406**	**KT750496**	**KT750596**	**KT750696**
Haemoproteus parabelopolskyi	**H_SYBOR01**	***Sylvia borin* (MLW_4144)**	**Malawi**	**KM056410**	**KT750470**	**KT750617**	**KT750723**
Haemoproteus belopolskyi	**H_MW1**	***Acrocephalus cinnamomeus* (MLW_3507)**	**Malawi**	**KM056408**	**KT750550**	**-**	**KT750650**
**Parahaemoproteus thraupi*	PIOLI01	*Piranga olivacea*	United States	AF465583	-	-	-
**Parahaemoproteus sp.*	P146	*Pycnonotus xanthopygos*	Israel	DQ451410	EU254598	-	EU254651
**Parahaemoproteus belopolskyi*	P60	*Sylvia curruca*	Israel	DQ451408	EU254603	EU254710	EU254657
**Parahaemoproteus coatneyi*	1060	*Dendroica coronata*	United States	EU254550	EU254595	EU254704	EU254648
**Parahaemoproteus fringillae*	169	*Zonotrichia albicollis*	United States	EU254558	EU254604	EU254711	EU254658
**Parahaemoproteus ilanpapernai*	P92	*Strix seloputo*	Singapore	DQ451424	EU254591	EU254700	EU254643
**Parahaemoproteus magnus*	P15	*Fringilla coelebs*	Israel	DQ451426	EU254594	EU254703	EU254647
**Parahaemoproteus passeris*	P38	*Passer moabiticus*	Israel	EU254554	EU254599	EU254708	EU254653
**Parahaemoproteus picae*	1543	*Picoides pubescens*	United States	EU254552	EU254597	EU254706	EU254650
Parahaemoproteus sp.	**H_AFR252**	***Camaroptera brevicaudata* (MOZ11_085)**	**Mozambique**	**KT750370**	**KT750462**	**KT750623**	**KT750730**
Parahaemoproteus sp.	**H_AFR253**	***Nectarinia olivacea alfredi* (MOZ11_105)**	**Mozambique**	**KT750371**	**KT750461**	**-**	**KT750731**

**Haemoproteus columbae*	P2111	*Columba livia*	United States	EU254548	-	EU254699	EU254642
**Haemoproteus turtur*	P143	*Streptopelia senegalensis*	Israel	DQ451425	EU254592	-	EU254644
****Haemoproteus sp.***	**H_AFR44**	***Turtur chalcospilos* (MLW_3595)**	**Malawi**	**KM056450**	**KT750491**	**-**	**KT750701**
****Haemoproteus sp.***	**H_AFR109**	***Columba arquatrix* (MLW_3900)**	**Malawi**	**KM056421**	**-**	**KT750611**	**KT750717**
****Haemoproteus sp.***	**H_AFR112**	***Columba arquatrix* (MLW_3909)**	**Malawi**	**KM056423**	**KT750475**	**KT750613**	**KT750719**
Leucocytozoon (Aves: Accipitriformes)							
**Leucocytozoon sp.*	157	*Accipiter brevipes*	Israel	EU254520	EU254565	EU254665	EU254611
**Leucocytozoon sp.*	2109	*Buteo jamaicensis*	United States	EU254518	EU254563	EU254663	EU254609
**Leucocytozoon sp.*	2208	*Buteo lineatus*	United States	EU254519	EU254564	EU254664	EU254610
**Leucocytozoon toddi*	L_ACCFRA01	*Accipiter francesiae*	Africa	AY684973	-	-	-
**Leucocytozoon mathisi*	L_ACCOP01	*Accipiter cooperii*	United States	HF543612	-	-	-
Plasmodium (Reptilia: Squamata)							
**Plasmodium azurophilum*	Red5686	*Anolis oculatus*	Hispaniola	EU254532	EU254575	EU254677	-
**Plasmodium chirichuahae*	NA	*Scleropus jarrovi*	United States	AY099061	KF049536	-	KF049558
**Plasmodium floridense*	NA	*Anolis spp.*	Dominica	EF079654	EF079654	EU254675	EU254620
**Plasmodium giganteum*	G6	*Agama agama*	Ghana	EU254534	EU254577	EU254679	EU254624
**Plasmodium hispaniolae*	DR490	*Anlolis sp.*	Dominican Republic	JN187905	JN187868	-	-
**Plasmodium lacertiliae*	CCA2227	*Emoia longicaudata*	Papua New Guinea	EU834709	EU834714	-	-
**Plasmodium leucocytica*	DR302	*Anolis cybotes*	Dominican Republic	JN187892	JN187865	-	-
**Plasmodium mexicanum*	E1	*Scleropus occidentalis*	United States	EU254529	EU254572	EU254674	EU254619
**Plasmodium minuoviride*	CCA0640	*Prasinohaema prehensicauda*	Papua New Guinea	EU834703	EU834711	-	-

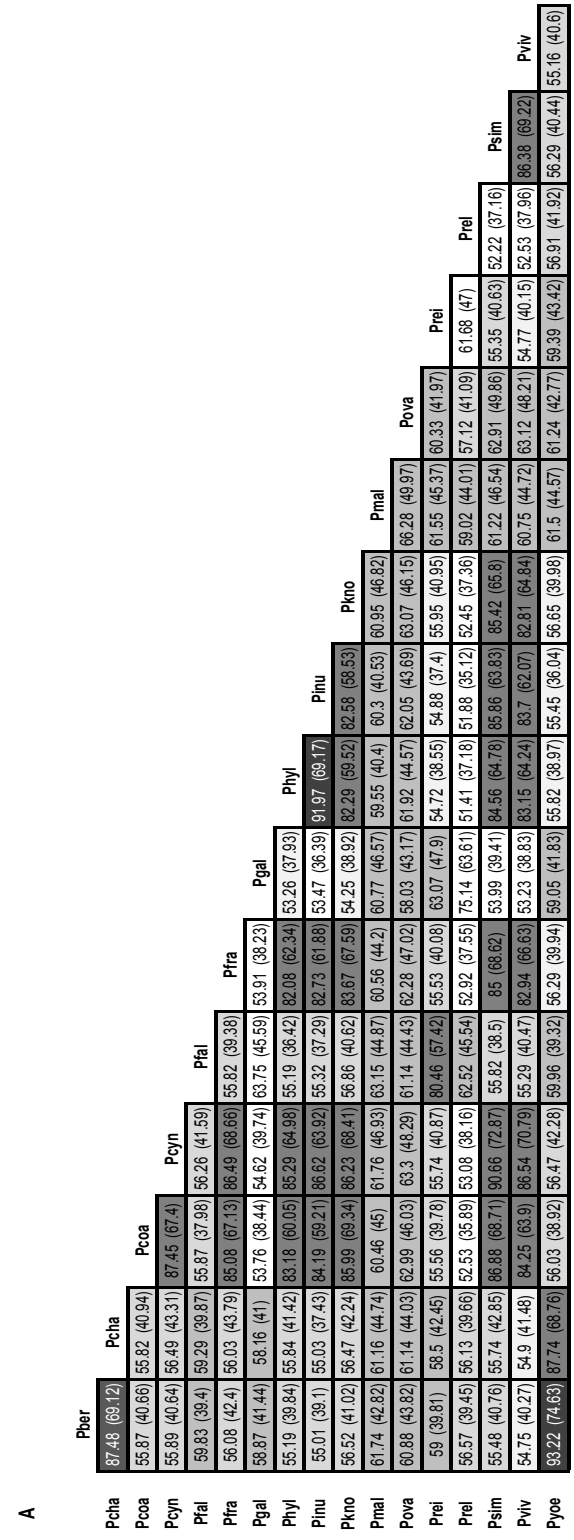


APPENDIX K

BEAST maximum clade credibility tree, based on four runs of 150 million generations with sampling every 5,000th tree (10% burn-in, 108,000 trees sampled, ESS > 1,000). Posterior probabilties are indicated next to nodes. Posterior probabilities < 0.50 are occasionally not shown due to spatial limitations.

APPENDIX M

PAIRWISE PERCENT IDENTITY BETWEEN *PLASMODIUM* TAXA FOR TWO VARIABLE SURFACE ANTIGEN CODING GENES, A) MSP-1, B) EMA)



A	Pber	Pcha	Pcoa	Pcyn	Pfal	Pfra	Pgal	Phyl	Pinu	Pkno	Pmal	Pova	Prei	Prel	Psim	Pviv
Pcha	87.48 (69.12)															
Pcoa	55.87 (40.66)	55.82 (40.94)														
Pcyn	55.89 (40.64)	56.49 (43.31)	87.45 (67.4)													
Pfal	59.83 (39.4)	59.29 (39.87)	55.87 (37.98)	56.26 (41.59)												
Pfra	56.08 (42.4)	56.03 (43.79)	85.08 (67.13)	86.49 (68.66)	55.82 (39.38)											
Pgal	58.87 (41.44)	58.16 (41)	53.76 (38.44)	54.62 (39.74)	63.75 (45.59)	53.91 (38.23)										
Phyl	55.19 (39.84)	55.84 (41.42)	83.18 (60.05)	85.29 (64.98)	55.19 (36.42)	82.08 (62.34)	53.26 (37.93)									
Pinu	55.01 (39.1)	55.03 (37.43)	84.19 (59.21)	86.62 (63.92)	55.32 (37.29)	82.73 (61.88)	53.47 (36.39)	91.97 (69.17)								
Pkno	56.52 (41.02)	56.47 (42.24)	85.99 (69.34)	86.23 (68.41)	56.86 (40.62)	83.67 (67.59)	54.25 (38.92)	82.29 (59.52)	82.58 (58.53)							
Pmal	61.74 (42.82)	61.16 (44.74)	60.46 (45)	61.76 (46.93)	63.15 (44.87)	60.56 (44.2)	60.77 (46.57)	59.55 (40.4)	60.3 (40.53)	60.95 (46.82)						
Pova	60.88 (43.82)	61.14 (44.03)	62.99 (46.03)	63.3 (48.29)	61.14 (44.43)	62.28 (47.02)	58.03 (43.17)	61.92 (44.57)	62.05 (43.69)	63.07 (46.15)	66.28 (49.97)					
Prei	59 (39.81)	58.5 (42.45)	55.56 (39.78)	55.74 (40.87)	80.46 (57.42)	55.53 (40.08)	63.07 (47.9)	54.72 (38.55)	54.88 (37.4)	55.95 (40.95)	61.55 (45.37)	60.33 (41.97)				
Prel	56.57 (39.45)	56.13 (39.66)	52.53 (35.89)	53.08 (38.16)	62.52 (45.54)	52.92 (37.55)	75.14 (63.61)	51.41 (37.18)	51.88 (35.12)	52.45 (37.36)	59.02 (44.01)	57.12 (41.09)	61.68 (47)			
Psim	55.48 (40.76)	55.74 (42.85)	86.88 (68.71)	90.66 (72.87)	55.82 (38.5)	85 (68.62)	53.99 (39.41)	84.56 (64.78)	85.86 (63.83)	85.42 (65.8)	61.22 (46.54)	62.91 (49.86)	55.35 (40.63)	52.22 (37.16)		
Pviv	54.75 (40.27)	54.9 (41.48)	84.25 (63.9)	86.54 (70.79)	55.29 (40.47)	82.94 (66.63)	53.23 (38.83)	83.15 (64.24)	83.7 (62.07)	82.81 (64.84)	60.75 (44.72)	63.12 (48.21)	54.77 (40.15)	52.53 (37.96)	86.38 (69.22)	
Pyoe	93.22 (74.63)	87.74 (68.76)	56.03 (38.92)	56.47 (42.28)	59.96 (39.32)	56.29 (39.94)	59.05 (41.83)	55.82 (38.97)	55.45 (36.04)	56.65 (39.98)	61.5 (44.57)	61.24 (42.77)	59.39 (43.42)	56.91 (41.92)	56.29 (40.44)	55.16 (40.6)

B	Pber	Pcha	Pcyn	Pfal	Pkno	Prei	Prel	Pviv
Pcha	94.86 (92.38)							
Pcyn	68.14 (52.55)	68.59 (53.42)						
Pfal	74.51 (57.22)	74.48 (56.95)	65.97 (46.09)					
Pkno	68.5 (51.53)	68.75 (52.21)	90.73 (84.48)	65.97 (44.98)				
Prei	74.71 (58.06)	74.48 (57.56)	66.07 (46.15)	97.29 (90.56)	66.12 (45)			
Prel	76.2 (62.08)	76.12 (62.49)	67.3 (49.94)	75.51 (56.2)	67.42 (49.72)	75.95 (55.83)		
Pviv	66.85 (49.93)	67.46 (50.93)	93.22 (88.91)	64.58 (43.79)	88.75 (81.99)	64.54 (43.82)	66.26 (47.66)	
Pyoe	96.98 (94.78)	94.77 (91.49)	68.54 (52.75)	75.02 (57.2)	68.95 (51.62)	75.34 (58.09)	76.51 (61.76)	67.44 (50.24)